FUNDAMENTALS OF BIO-CHEMISTRY

in relation to Human Physiology

BY

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Foreword

WHILE the study of physiological chemistry has been adequately dealt with from the *practical* point of view, the *theoretical* treatment of the subject seems still to be confined to larger treatises containing a much greater wealth of detailed information than is appropriate in an elementary introduction suitable for readers new to the subject. It appeared desirable, therefore, to attempt to describe in a continuous story the more important generally-accepted principles which have been derived from the study of the chemical changes occurring in the human body. The present volume represents the result of such an attempt. During its preparation I have borne in mind the particular needs of my own students, but, as I have demanded the minimum of previous knowledge of pure chemistry and physics, it is hoped that the book may prove not too unsuitable to a wider circle of readers.

Although the work is intended to constitute the most elementary of introductions to the subject, I have not hesitated to indicate the sources where further information can be obtained on the topics discussed. This procedure is justified, I think, even in so small a volume, for even the elementary student can gain considerable help and inspiration from the reading of only a few significant sentences from the original work of an authority, although he be unable to follow the more intricate portions of the argument. And, further, it is only when the student comes to realise that one single book can never truly suffice for the intelligent reading of any subject, but must be supplemented by the comparison and sifting of information

gleaned from as many sources as are available to him, that he can hope to make his knowledge so intimate a part of his mental equipment that it will be ready to serve him whenever he needs it, in either a professional or an academic career. The student who would *realise* his subject must turn often to the original sources of its material; he will then come to regard it not as being a kind of inborn inspiration given only to the few, but—as it really is—as being made up of the lasting results of human intellectual effort, welded into a whole which never becomes complete or flawless, but which is always being increased and perfected and ordered by the labour of men's minds. . . .

It has been my desire that any merit my book may possess may result from its containing *less* of information rather than more than other books contain. But I am fully aware that my anxiety for the omission of detail must often have run to such excess as to constitute a fault rather than a virtue. That I have not erred further in this direction is due to the kindly help I have received during my task from Professor F. G. Hopkins and Mr. S. W. Cole, to both of whom I tender my best thanks.

I wish also to thank Mr. John Murray for his courteous permission to reproduce three of the coloured diagrams of spectra from Halliburton's "Physiology."

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CAMBRIDGE,
Autumn, 1922.

Contents

CHAPTER I.

THE NATURE OF LIVING MATTER: THE CONSTITUTION OF THE PROTEINS.

"We are such stuff
As dreams are made on, and our little life
Is rounded with a sleep."
—*The Tempest.*

WE cannot begin our study of the chemical changes occurring in the living organism more suitably than by considering the properties and behaviour of the most important and characteristic substances which enter into the composition of all its cells and tissues—to wit, the **proteins.** Of course, in that complex system which makes up a living tissue there are contained, in addition to the proteins, many other substances such as starches, sugars, fats, salts and so on, but these are of secondary importance, and represent, roughly, reserve materials waiting to be used by the living structure rather than fundamental parts of that structure itself. In other words, all that association of phenomena which we term life is manifested only by matter which is made up to a very large extent of proteins, and is never exhibited in the absence of these substances. But the investigation of the proteins has proved to be a very difficult task. In general, of course, when the chemist wishes to study the

properties and constitution of any substance he endeavours first of all to prepare the substance in a pure condition by distillation, if it be a liquid, or by oft-repeated crystallisation if it be a solid. In the case of the proteins, however, we have to deal with substances which are extremely liable to change. Indeed, if they did not possess this well-marked changeableness of nature they would not be suited to form the chief constituents of living tissues whose every manifestation is in essence a change,—a change from rest to activity, from youth to age, from health to disease and death. . . . . .

But it is just this liability to change which is the chief source of difficulty in the chemical study of the proteins. Take a solution of a protein, heat it but a comparatively few degrees above the normal temperature of the body, and irreversible processes of decomposition set in; make the solution a little too acid, or too alkaline, and, once more, the original protein is broken up and lost. One has but to think of the properties of egg-white—which is composed chiefly of the protein egg albumin—to picture to oneself its viscid, slimy, non-crystallising solution which coagulates to an opaque white mass as a result of a relatively insignificant rise of temperature—in order to realise the serious nature of the obstacles which the properties of the typical proteins oppose to the investigation of their molecular structure. Certainly egg albumin is one of the few proteins which have been obtained in a crystalline condition, but this achievement is possible only by the employment of very accurately controlled process of precipitation, too limited in their application to be used as routine methods of purification. The ordinary condition of proteins in solution is not one which is adapted for the fashioning

of the exquisite architecture of a crystal. But while there thus exist enormous difficulties in the obtaining of most individual proteins in a condition of sufficient purity to ensure that their ultimate analysis by the accurate combustion methods employed by the organic chemist shall yield results possessing more than a rough, hardly quantitative, significance, a very considerable amount of information with regard to the chemical constitution of the proteins has been derived from a study of the products obtained by disrupting their molecules. It is a case where the structure is too intricate and too delicate for us to examine as a whole by our present clumsy methods: all we can do is to try to form some picture of the original complex by studying the fragments which remain when the destructive tendencies of our natures have been given their freedom to perform their worst upon it. The easiest way in which a protein can be broken down into simpler and more easily investigated substances is by boiling it with a dilute acid. Suppose, for example, that we take some egg-white and boil it for some hours in a reflux apparatus with dilute hydrochloric acid, then gradually the properties typical of egg-white disappear from the mixture and we are left with a solution containing the breakdown products of the egg albumin. And when we examine these products left in the solution we find that, apart from a certain amount of ammonia and small quantities of unidentified substances, the protein has been broken down completely into a mixture of amino-acids. We must pause for a moment to remind the reader exactly what an amino-acid is. He is probably quite familiar with the series of saturated fatty acids beginning with formic acid, H.COOH. The second member of the series is acetic acid, CH_3.COOH,

and the third, propionic acid, $CH_3.CH_2.COOH$—a name which means "first fat," and which refers to the fact that while formic and acetic acids are pungent-smelling liquids miscible with water in all proportions, propionic acid has only a limited solubility, so that excess of it remains as an oily layer. For the present we will mention only the next member of the series, namely, butyric acid, $CH_3.CH_2.CH_2.COOH$, which occurs in the fat of butter.

Now an amino-acid is simply an acid in whose molecule a hydrogen atom of the radicle has been replaced by an amino-group—NH_2. We say a hydrogen atom of the radicle because, of course, the—COOH group must remain intact as it is the characteristic group in the molecules of these, and of most, organic acids. The amino-acids which are of interest to us at present as being the more important products of the breakdown of protein molecules are all amino-derivatives of these fatty acids. Thus from acetic-acid we obtain amino-acetic acid, $NH_2.CH_2.COOH$, which is usually called **glycine,** sometimes glycocoll, on account of its sweet taste. When we come to consider the formula of propionic acid we at once realise that we can insert the amino-group in the place of one of the hydrogen atoms of the CH_2 group thus:—$CH_3.CHNH_2.COOH$, or in place of one of the hydrogen atoms of the terminal CH_3—group, so:—$NH_2.CH_2.CH_2.COOH$.

We have thus theoretically two distinct amino-propionic acids differing in chemical structure and properties. Both these substances are, in fact, known. In order to distinguish between such closely related substances and to indicate the position of the amino- or other substituting group in the molecule, a convention is adopted whereby the carbon atoms in a molecule containing a long straight chain are labelled in order with the letters of the Greek

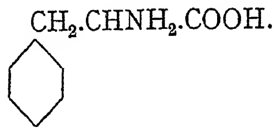
alphabet. Furthermore it is agreed to commence this labelling with the carbon atom next to the characteristic group in the molecule. In the case of an acid this is the —COOH group, so that, according to this nomenclature, the atoms in the molecule of propionic acid will be designated thus:—

$$\overset{\beta}{CH_3}.\overset{\alpha}{CH_2}.COOH.$$

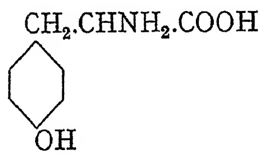
For a longer chain, of course, further Greek letters are employed. We refer, therefore, to the two amino-propionic acids respectively as α-amino-propionic acid $CH_3.CHNH_2.COOH$, and β-amino-propionic acid $NH_2.CH_2.CH_2.COOH$. Now while both these substances are of more or less equal interest to the organic chemist, the bio-chemist has no interest in the β-compound for it does not occur amoug the substances contained in or derived from living organisms. In contrast with this, α-amino-propionic acid is of such extreme importance from our point of view, that it is given a special name, **alanine.** As a matter of fact it is found that all the amino-acids which are breakdown products of proteins are *alpha*-amino-acids—the amino-group is always attached to the carbon atom next the carboxyl group. There are amino-acids which contain more than one amino-group—the di-amino acids for instance—and in these we have amino-groups attached to other carbon atoms as well: but, even so, one amino-group always occupies the α position.

To return to the amino-acid alanine,—this is an important substance, not only because it itself occurs very commonly as a constituent of protein molecules, but because also it has several derivatives which are important for the same reason. If we introduce into the molecule

of alanine a phenyl group,* C_6H_5—in the β position, we obtain phenyl alanine:—



This, again, is a substance of frequent occurrence as a protein constituent. By the replacement of the hydrogen atom understood to be attached to the carbon atom in the opposite or "para" position to the side chain, we arrive at the formula form α-amino—β-hydroxy-phenyl-propionic acid:—

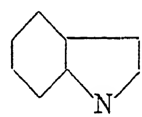


the more familiar name of which is **tyrosine**—a word which, being derived from the Greek *τυρος* meaning "cheese," serves to remind us that the particular amino-acid we are considering was first obtained (by Liebig) by heating up cheese with potash. The making of Welsh

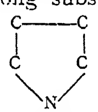
* It should be hardly necessary to remind the student that the six carbon atoms in the phenyl group are arranged in a ring which is usually represented as a hexagon. Each corner of the hexagon then represents a carbon atom, and to each carbon atom not represented as being attached to any other group a hydrogen atom is understood to be linked. Further, there exist not only rings composed of six carbon atoms, but also, though less commonly, those of five or even four. And not only do we know these varieties of rings composed entirely of carbon atoms, but there are also those which contain one or more atoms of some other element. Nitrogen atoms take a place in many such heterocyclic rings, and whenever they do so it is necessary to write the "N" representing the nitrogen atom across the corner of the ring and not outside it, otherwise it would appear that the nitrogen formed part of a side chain attached

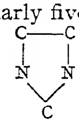
rarebit in this fashion has never been popularised, but sufficient attention has been given to it to make it evident that in Liebig's experiment there occurred a breaking down of the chief protein of the cheese, namely **casein,** into its constituent amino-acids, of which tyrosine happened to be the one most readily isolated from the melt.

There are other ring derivatives of alanine which occur among the constituents of the protein molecule. On page 6 (footnote) we reminded the reader that many conjugated ring structures were known. Of these there is one known as the indol ring, which is composed of a benzene ring and the heterocyclic pyrrol ring:—



to an understood carbon atom, instead of being a constituent atom of the ring itself. Thus two very important heterocyclic rings which we meet among substances of biological importance are the

pyrrol ring  containing one nitrogen atom

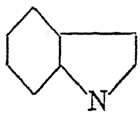
and four of carbon, and the **iminazol ring** which is similarly five-membered, but contains two nitrogen atoms thus:— 

Lastly we have substances whose molecules are composed of two rings having two atoms in common. Of these the most familiar is probably naphthalene—the so-called "camphor," which is extensively used to ward off moths from clothes. Its formula is represented

as two benzene rings with two carbon atoms in common:—

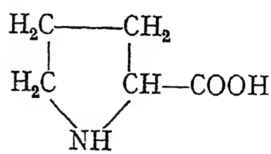
But we shall also meet substances in which the two fused rings are not similar as in this case

When this is substituted in the β-position in the molecule of alanine, α-amino-β-indol-propionic acid results:—



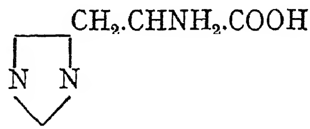
$CH_2.CHNH_2.COOH.$

This substance is known also as **tryptophane,** because it is the substance which is responsible for the bright violet colour which is given by a pancreatic or tryptic digest of a protein when bromine water is added to it (Greek $\phi\alpha\nu o\varsigma$ = bright). It was first isolated from the products of protein digestion by Hopkins and Cole in Cambridge in 1901. While these workers were engaged upon this task, news arrived from Berlin that Emil Fischer was also isolating a new product of protein hydrolysis. Furthermore, Fischer's new substance was found to be a white crystalline solid, just as was the product obtained by Hopkins and Cole. There was, therefore, for a time, some doubt as to which of the great schools of bio-chemistry would be able to claim priority for the discovery of tryptophane, until, when Fischer published his results, it was seen that the compound he had obtained was not tryptophane, nor, indeed, a true amino-acid at all, but a substance called **proline** whose formula is as follows:—



We must mention one other important derivative of alanine which contains in the β-position a five-membered ring containing two nitrogen atoms and known as the

iminazol ring. This iminazol-alanine then has the formula:—



It is the amino-acid more familiarly known as **histidine.**

We have now mentioned sufficient typical amino-acids to give an idea of the range of variation of structure found in the group. We need, however, to consider in more detail the relationship between these amino-acids and the fully formed protein molecule. The fact is that if we take *any* protein and disrupt its molecule by boiling with a dilute acid we find that we obtain a mixture of amino-acids, all of which are α-amino-acids, and among which are included all those we have already mentioned, together with about a dozen more. Of these remaining ones we shall describe a few more in detail later. This decomposition into amino-acids is found to occur with all proteins: we may say, therefore, that the amino-acids are the units of which protein molecules are composed: that they represent, so to speak, the bricks of which the complicated edifices are built. We have said that the majority of proteins give the same score, or so, of amino-acids on decomposition. But the various proteins yield these same amino-acids often in widely differing proportions, so that we may say that while all protein molecules consist of complexes of amino-acid molecules, the ratio in which these constituents are combined in one protein is very different from that in which these same substances occur in a second. In special cases a particular protein may contain an abundance of a

given amino-acid, another protein may contain almost none of it.

A knowledge of this general principle gives the key to the whole of the later study of the behaviour of proteins in the body. It also has important bearings on a topic which we will now discuss, namely, the qualitative tests for proteins. It is evident that it is of the utmost importance to be able to decide whether or no any given biological fluid contains a protein, and some of the most valuable evidence on this question is furnished by certain colour reactions which proteins give. Of these we will describe that known as **Millon's reaction** first. If a quantity of Millon's reagent be added to a solution containing a protein a white precipitate is formed, and this precipitate turns a brick red colour on boiling. It has been found that this colour reaction is given also by tyrosine, and, indeed, by all substances which, like tyrosine, contain the oxy-phenyl ring. In fact, the reason why practically every protein gives Millon's test is that tyrosine is of almost universal occurrence in proteins. A protein which contains no tyrosine will therefore not give Millon's reaction. Such a protein is **gelatine,** which in the pure condition does not give the test, although the commercial product is usually contaminated with traces of some impurity which does give a faint reaction. Incidentally, we must call attention to the fact that it is absolutely necessary that a red colour should be produced on boiling, the white precipitate not occurring under all conditions. And again many substances of physiological importance, including even urea, give with Millon's reagent a white precipitate, which, however, does not show the colour change on heating. In order to understand this we must know that Millon's reagent is a mixture of the

nitrates of mercury, obtained by dissolving the metal in strong nitric acid. It also contains excess of the nitric acid and a variable quantity of nitrous acid formed by the reduction of the nitric. Now when urea is added to solutions of mercury salts, white precipitates, composed of basic double salts of urea and mercury, are precipitated. This accounts for one aspect of the behaviour of urea towards Millon's reagent. But there is a second; for whenever a substance containing the amino-group is brought into contact with nitrous acid, a reaction occurs which leads to the liberation of nitrogen. In the particular case of urea this takes place as follows:—

$$\underset{\text{urea + nitrous acid}}{O{=}C\Big\langle\begin{matrix}\text{HO}\,\vdots\,\text{N}\,\vdots\,\text{O}\\ \vdots\,\text{N}\,\vdots\,\text{H}_2\\ \vdots\,\text{N}\,\vdots\,\text{H}_2\\ \text{HO}\,\vdots\,\text{N}\,\vdots\,\text{O}\end{matrix}} \longrightarrow \underset{\text{carbonic acid}}{O{=}C\Big\langle\begin{matrix}\text{OH}\\ \text{OH}\end{matrix}} + 2N_2 + 2H_2O$$

carbonic acid being formed, and further decomposing into carbon dioxide and water. We do not wish to elaborate this matter further than to point out that the mere production of a white precipitate with Millon's reagent is no certain test for the presence of tyrosine or a protein unless a red colour appears on boiling, and that if, instead, the reaction is accompanied by effervescence, then it is probable that urea may be present.

A second colour test given by the proteins is that known as the **glyoxylic reaction.** The reagent is prepared by reducing oxalic acid with sodium amalgam or magnesium powder. There is thus obtained a solution whose essential constituent is glyoxylic acid, $\begin{matrix}\text{CHO.}\\ \text{COOH}\end{matrix}$ A quantity

of this "reduced oxalic acid" or "glyoxylic reagent" is mixed with some of the solution to be tested. Then a layer of strong sulphuric acid is allowed to run down the side to the bottom of the test-tube, and if a protein be present a purple ring forms at the junction of the two layers of liquid. This reaction is associated with the presence of tryptophane, and indeed, the first isolation of tryptophane was the result of a systematic search for the substance in the protein molecule, which was responsible for the formation of the purple colour under these conditions. Naturally, then, any protein from whose molecule tryptophane is absent will fail to give this test, and again we find that this is the case with gelatine. It gives absolutely no colour with glyoxylic acid in the presence of strong sulphuric acid. The student will have realised by this time that these two so-called colour tests for proteins are simply tests for the amino-acids tyrosine and tryptophane, and that these reactions can be used as tests for proteins merely because tyrosine and tryptophane are of such common occurrence in protein molecules.

It is the presence of aromatic ring structures such as are found in tyrosine, tryptophane and phenyl-alanine which is responsible for the **xanthoproteic** reaction which is given by all proteins. Strong nitric acid forms yellow nitro-derivatives of these ring structures, the colour of which is turned to orange on addition of ammonia. The first of these facts is a matter of everyday experience to anyone who allows drops of strong nitric acid to come into contact with the proteins of his skin. Gelatine responds to the xanthoproteic test owing to the presence of phenyl-alanine.

We turn now to consider the question as to the manner in which amino-acids are combined to form protein

molecules. Since the amino-group and the carboxyl group of an amino-acid are the two most reactive groups in the molecule it would be expected that linkage of amino-acid molecules would be brought about by means of these two groups. Evidence that this is the case is furnished by a number of observations. For example, amino-acids, like other substances containing the $—NH_2$ group, evolve nitrogen when treated with nitrous acid. Now when a protein is treated with this reagent very little nitrogen is evolved: this indicates that the amino-groups of the constituent amino-acids are not free in the protein molecule, and that they are therefore concerned in forming the linkages which serve to unite these units in the whole complex. And, indeed, Emil Fischer has succeeded in causing amino-acid molecules to unite to form long complex chains by the elimination of water between the amino-group of one molecule and the carboxyl group of the next. In practice this result has to be achieved by somewhat roundabout methods, but this circumstance does not affect our present argument. Let us take the case of the condensation of a molecule of alanine with one of glycine:—

$$\underset{\text{alanine}}{CH_3.CHNH_2.CO}\boxed{OH\ \ H}\underset{\text{glycine}}{HN.CH_2COOH}$$

$$\longrightarrow \underset{\text{alanyl-glycine}}{CH_3.CHNH_2.CO—HN.CH_2.COOH}$$

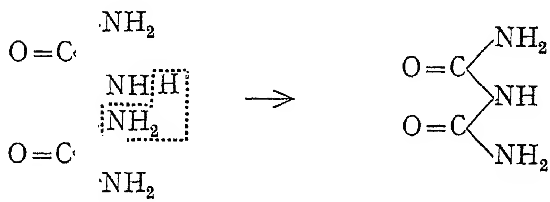
In the resulting product it will be seen that there is still an amino-group and still a carboxyl group both of which are available for forming linkages, in a precisely similar manner with other amino-acid molecules, so that by a repetition of the appropriate chemical manipulations

compounds whose molecules consist of chains of amino-acids can be built up.

From this mode of formation it will be seen that the characteristic group whereby the amino acid molecules become linked together is —CO—NH—. This is called the **peptide linkage,** and the substances prepared in the way we have described are referred to as di-, tri-, tetra-, or polypeptides, according to the number of amino-acids which are united in their molecules. Many of these **polypeptides** have now been prepared, and it is found that the more amino-acids which can be condensed in a single long polypeptide chain the more nearly does the resulting compound resemble in properties the simpler of the naturally occurring peptones. This is as far as it has been found possible, up to the present, to proceed with the synthesis of protein molecules. But there is no reason to doubt that further progress will be achieved when yet more efficient methods of bringing about this condensation are devised, and also, probably more important still, when we possess information which will enable us to choose from among the enormous numbers of possible arrangements of these amino-acid molecules, those which actually occur in the structure of the proteins. Even so, sufficient advance has been made to justify the conclusion that, in general the various proteins are larger or smaller chemical complexes of amino-acids between which the chief connecting group is the peptide linkage.

A third reaction which is important as a means of detecting the presence of proteins is the formation of intensely-coloured copper derivatives—usually blue or purplish, but in some cases pink—when a drop of dilute copper sulphate solution is added to a protein solution

made strongly alkaline with excess of caustic soda. This is known as the **biuret test** because it was first used as a test for biuret—a substance produced by heating solid urea, two molecules of which react with the evolution of ammonia:—



urea biuret

But this colour reaction is by no means confined to biuret itself; it has been found to be given by practically all compounds which contain two—CO—NH—groups united to the same carbon (or nitrogen) atom. This, of course, includes biuret itself, in which a nitrogen atom unites the two—CO—NH—groups, and practically all the proteins and polypeptides, where the union is brought about by a carbon atom. Naturally gelatine in common with all other proteins contains many such peptide linkages, and in virtue of this it gives the biuret reaction well.

We have explained the evidence on which is based the view that the protein molecule is composed of a complex of amino-acids condensed together by the elimination of water. It is now easy to see that the reaction which occurs when a protein is heated in acid solution is the reverse of condensation: it is a process of hydrolysis in which water molecules are added to the peptide linkages and the free amino-acids are re-formed. Taking our original example of alanyl-glycine we may write:—

$$CH_3.CHNH_2CO\text{-}\boxed{OH\ |\ H}.NH.CH_2COOH$$

alanyl-glycine

$$\longrightarrow CH_3.CHNH_2.COOH + NH_2.CH_2.COOH$$

alanine glycine

Before we turn from this question of the chemical structure of the protein molecule we must mention that practically all proteins contain sulphur. This sulphur is present in the form of a sulphur containing amino-acid called **cystine.** We shall understand the formula for this substance most easily if we remember that a divalent sulphur atom can often replace an oxygen atom in a compound and so give rise to a thio-derivative. Among organic compounds we have such as:—

$C_2H_5.OH$	$C_2H_5.SH$
Ethyl Alcohol	Thio alcohol—also called "**Mercaptan**" because it readily attacks mercury ("mercurium captans.")

Now in a similar way the β-oxy-derivative of alanine, namely

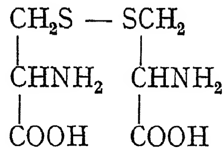
$$\begin{array}{l} CH_2OH \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$

has a corresponding thio-derivative with the formula

$$\begin{array}{l} CH_2SH \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$

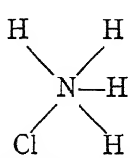
This is called cyst*ein.* By the removal of the hydrogen atom from the sulphydryl group of each of two molecules of cystein and union of the two resulting residues we

obtain the complex sulphur containing amino-acid cystine itself:—

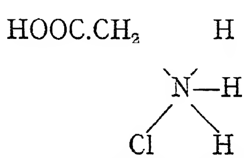


The presence of sulphur in a protein can be readily demonstrated by boiling it with strong caustic soda solution. Sodium sulphide is then produced and can be detected by the addition of a solution of a lead salt, when insoluble black lead sulphide is precipitated.

Before we leave the study of the amino-acids we must refer to one of their most characteristic chemical properties. By virtue of the fact that they contain the carboxyl group they behave as acids and form salts with bases. For example, we can readily obtain the sodium salt of glycine, $NH_2.CH_2.COONa$. But these compounds also contain the amino-group which is basic in character and confers upon the substances containing it the property of acting as bases and forming salts with acids. Thus glycine reacts with hydrochloric acid form a hydrochloride of the formula $HCl.H_2N.CH_2.COOH$, which is a kind of derivative of ammonium chloride thus:—



ammonium chloride



glycine hydrochloride

A substance which possesses this property of acting as a base towards acids and as an acid towards bases is said to be **amphoteric.** Whole protein molecules are also

amphoteric, because in them not quite all the amino- and carboxyl-groups are used to form peptide linkages so that a few remain free.

SUMMARY.

We may now summarise the contents of this chapter in a schematic form thus:—

Proteins are the characteristic constituents of living matter. Their molecules are built up of large numbers of amino-acids united by peptide linkages. Of these amino-acids, important examples are:—

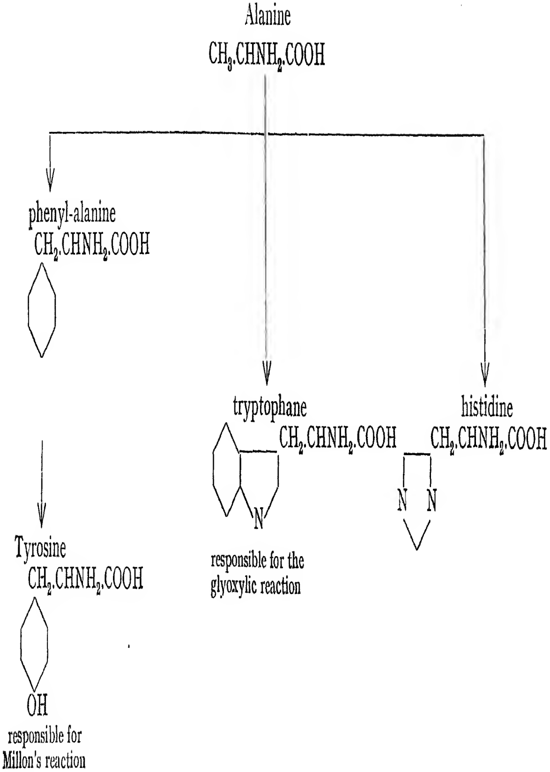
amino-acetic acid, glycine:—$NH_2.CH_2.COOH$,
α-amino-propionic acid, alanine:—$CH_3.CHNH_2.COOH$,

with its derivatives phenyl-alanine, tyrosine, tryptophane and histidine, whose relationships are shewn on the next page.

The sulphur contained in the protein molecule is mostly in the form of the complex thio-amino-acid cystine:—

$$\begin{array}{lcl} CH_2S & - & SCH_2 \\ | & & | \\ CHNH_2 & & CHNH_2 \\ | & & | \\ COOH & & COOH \end{array}$$

The biuret reaction given by proteins is due to the presence of the linkage –CO–NH–C–CO–NH–.


Alanine
CH3.CHNH2.COOH
phenyl-alanine
CH2.CHNH2.COOH
tryptophane
CH2.CHNH2.COOH
N
histidine
CH2.CHNH2.COOH
N N
responsible for the
glyoxylic reaction
Tyrosine
CH2.CHNH2.COOH
OH
responsible for
Millon's reaction

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The student can obtain any further information he may desire with regard to the various amino-acids and the structure of the protein molecule from Plimmer's monographs:

The Chemical Constitution of the Proteins. Part I. Analysis (3rd edition, 1917). Part II. Synthesis (2nd edition, 1912.) (Monographs on Biochemistry; London: Longmans, Green & Co.)

Full details of the methods of carrying out the tests for proteins are given in

Practical Physiological Chemistry, by S. W. COLE. 6th edition, 1920. (Cambridge: W. Heffer & Sons, Ltd.)

The original account of the isolation of tryptophane by Hopkins and Cole is contained in a paper entitled:

A Contribution to the Chemistry of the Proteids. Part I. *A Preliminary Study of a hitherto undescribed Product of Tryptic Digestion. Journal of Physiology,* Vol. XXVII. (1901), p. 419.

This article should be read by all students as an example of the way in which such a problem is dealt with in research.

CHAPTER II.

THE CHIEF VARIETIES OF PROTEINS: THEIR PROPERTIES AND RELATIONSHIPS.

"This will teach you their names, the ingredients they're made of,
And which to indulge in, and which be afraid of."

Ingoldsby Legends.

WE have learnt in a general kind of way that a protein molecule is a complicated structure built up of amino-acid molecules. It is now necessary to point out that these chemical aggregates of amino-acids may vary very considerably in their complexity, so that there come to be a large variety of protein molecules ranging from those which contain comparatively few amino-acid molecules, and are relatively small and simple, to others whose molecules are enormous compounds of large numbers of these chemical units. With these varying degrees of complexity are associated well-marked differences of chemical and physical properties, which make it possible to classify this wide range of substances into subsidiary groups, and so to deal with them systematically and scientifically. The most important property which is utilised for the separation, and, indeed, in many cases the definition, of the chief classes of the proteins, is that of solubility. Our task in this chapter thus resolves itself into a study of the various kinds of proteins and of their solubilities, and of the method whereby this property is used in their identification. If Chapter I may be regarded from some points of view as supplying the answer to the questions "What is a protein?" and "How does one determine

whether there is a protein in a given mixture?" the present chapter may be said to carry the investigation further, and to indicate how to meet a demand for information as to the nature of a protein already known to be present.

Naturally, the most familiar proteins are those which are common to all living tissues, such as the group of similar proteins known collectively as the **albumins.** Of these we have already met one example, egg albumin. Also of widespread occurrence in all living matter we find the members of a second group of proteins called the **globulins.** These two groups of proteins include the great bulk of the proteins of most living tissues. They are therefore referred to as the "native" proteins. They are all very similar in chemical structure, but differ in this very important property of solubility. For while the albumins are soluble in distilled water, the typical globulins will not dissolve unless a certain small amount of sodium chloride or other neutral salt be present. So that when a salt solution containing globulins is dialysed, these substances are gradually precipitated as the salt is removed. Both albumins and globulins are easily soluble in dilute acid and alkaline solutions, but they differ again when their behaviour towards strong solutions of salts is considered. In concentrated solution, dissolved neutral salts seem to have a kind of attraction for the solvent water, so that they have the power, so to speak, of robbing the solvent from another dissolved substance such as a protein, and so of precipitating it. This is a general property of all neutral salts, but it is exhibited in the most marked degree by those which are most soluble, that is, those which can be obtained in greatest concentration in solution. For this reason ammonium sulphate

is most conveniently used for the purpose. It is found that if to a solution of a globulin there is added such an amount of ammonium sulphate as is just half of the quantity which would be required to saturate the solution, the globulin is precipitated by the withdrawal of the water. This "half-saturation with ammonium sulphate" is most easily performed by adding to a given volume of the protein solution an equal volume of a saturated solution of the salt. The globulin which is so precipitated has not undergone a chemical change, and will readily pass into solution again as soon as the excess of ammonium sulphate has been washed away from it. On the other hand, albumins are not precipitated unless the solution is *saturated* with ammonium sulphate, a condition which can be obtained only by shaking with the solid salt and not by the addition of any quantity of an already saturated solution. It is obvious that the addition in any amount of saturated ammonium sulphate solution to any liquid not already saturated with the salt can never result in a mixture in which the salt is present in saturation concentration. As a matter of detail we ought to mention that, although the behaviour we have described is shewn by the typical globulins, yet there are some, called the pseudo-globulins, which resemble the albumins in being soluble in distilled water. But they all differ from the albumins in being precipitated by half-saturation with ammonium sulphate. The albumins and globulins have in common the property of coagulating when their solutions are heated to a certain temperature. This distinguishes them from all other proteins, none of which are coagulable by heat. The process of coagulation results in a removal of the protein from solution in a manner which differs from the precipitation by

ammonium sulphate already mentioned inasmuch as the coagulation is accompanied by a partial decomposition of the protein molecule, which we shall describe later. This coagulation on boiling furnishes us with a method of separating albumins and globulins from all other proteins.

By the partial breaking down of the molecules of the native proteins by the action of acids or alkalies in the cold we obtain a group of simpler substances of smaller molecular weight, known as the **metaproteins.** These differ from the native proteins from which they are derived by being soluble only in dilute acid or alkaline solutions. They are precipitated on neutralisation, for neither pure water nor neutral salt solutions will dissolve them; and ammonium sulphate, even in half-saturated solution, will precipitate them. They are also produced by partial hydrolysis when albumins and globulins are heated with water, and indeed form the coagulum which these native proteins yield on heating. This explains why the process of coagulation is an irreversible one, for it has involved a conversion of the original albumins or globulins into metaproteins. Furthermore, we can now see that in order to remove a coagulable protein completely and clearly from solution, the coagulation must take place in approximately neutral solution. For in such a solution the metaprotein formed is insoluble. But if the solution be more acid or more alkaline than corresponds to the condition of minimum solubility of the metaprotein, this, though produced, will remain in solution, and no coagulum will be formed. The application of this fact to the analysis of protein solutions is evident. For example, it is useless to try this formation of a coagulum on boiling as a test for albumins in urine, without first

neutralising the fluid if it is appreciably acid or if it has become alkaline on standing. And, in general, if the reaction of the liquid under examination is not appropriate, then the metaprotein will not be completely carried down in the coagulum, and will give trouble at later stages of the analysis.

When the digestive enzyme pepsin of the gastric juice is allowed to act upon native proteins a partial disruption of their molecules occurs, and this takes place in a fashion somewhat similar to the splitting brought about by cold acids and alkalies, so that similar simple products are obtained. If we take a peptic digest we can resolve it into its constituents somewhat as follows: Firstly, we neutralise the mixture to precipitate metaproteins and then boil the filtrate in order to coagulate and remove any unchanged albumins or globulins. We then half saturate the solution with ammonium sulphate, and obtain a precipitated fraction known as **primary albumoses** or primary proteoses. Having filtered this off, we then fully saturate the filtrate with ammonium sulphate, and obtain a fraction which consists of **secondary albumoses.** Even after complete saturation with ammonium sulphate some proteins remain in solution. These are the **peptones.** They are the only proteins which can withstand the precipitating action of so strong a neutral salt solution as this, so that all other proteins may be separated from these by full saturation with ammonium sulphate. In other words, if a solution be saturated with ammonium sulphate and filtered, and if by means of the colour reactions a protein is found to be present in this filtrate, that protein must necessarily be a peptone.

Incidentally we must remind the student that several

of the protein colour reactions described in Chapter I, are due to the presence of particular amino-acids in the protein molecule. But the free amino-acids are much more readily soluble substances than the proteins; they are not precipitated by ammonium sulphate and other reagents which bring down the proteins, nor do amino-acids give the biuret reaction, for they contain no —CO–NH— group. This last fact serves to distinguish free amino-acids from peptones, which give a pink colour in the biuret reaction.

So far we have been passing from the complex to the simpler proteins. We shall now pass in the opposite direction to substances more complex than any that we have yet mentioned. There are many substances whose molecules consist of a compound of a complete protein molecule with some other substance which is not a protein. These substances are called **conjugated proteins.** A very familiar example is hæmoglobin—the red respiratory pigment of the blood. This consists of a typical globulin called globin, united to a non-protein molecule called hæmatin. In some conjugated proteins the non-protein part of the whole complex is phosphoric acid. In these cases we are dealing with the **phospho-proteins** of which casein, the chief protein of milk, is an example. Or again, the non-protein group may be a carbo-hydrate such as a sugar. In this case the substance belongs to the **gluco-proteins.** Mucin, the substance which gives the slimy lubricating properties to many mucous secretions, is an example of these. Lastly, we reach the height of complexity in the conjugated proteins present in the nuclei of cells which are termed **nucleo-proteins.** In these the non-protein half of the molecule is itself a complex of molecules of phosphoric

acid, sugars and basic substances related to uric acid termed purine bases. We do not wish to describe these nucleo-proteins in detail until we treat of their relation to uric acid in Chapter VII. Our main object at the moment is to point out that the solubility relationships of these three groups of conjugated proteins are all very similar. They are all soluble in dilute alkaline solutions, but in no other of the media we have previously mentioned. They are specially insoluble in dilute acids, so that a liquid whose reaction is acid cannot contain these in solution; and, further, they are precipitated on the change of reaction from alkaline to acid. Mucin is distinguished, however, from nucleo-proteins and casein by the fact that it is insoluble in strong acetic acid—a fact often utilised by the histologist for rendering more easily visible the mucin in cells.

Lastly, we must mention that exceptional or incomplete protein, gelatine, which, as is well known, is soluble enough in water, salt solutions, acids and alkalies if these are warmed, but its solutions, if sufficiently strong, gelatinise on cooling. It is precipitated by half saturation with ammonium sulphate.

This brings us to the end of our survey of the chief groups of substances included under the term proteins and of their solubility relationships. It remains merely to point out that it is by the utilisation of these different solubilities that proteins are separated and identified. This constitutes the information which it is essential to hold clearly in mind in order to achieve success in the solution of this problem. On account of its extreme importance we summarise on page 29 the solubilities we have already mentioned—in the form of a diagram in which a line drawn on a level with the name of a

particular protein and below that of one of the solvents indicates that that protein is soluble in the particular solvent, whereas, where the space is left blank the protein is insoluble in, or in the case of the ammonium sulphate solutions, is precipitated by, the particular medium. The student will realise that this scheme will tell him what proteins may be present in (say) an acid solution or in a solid mixture which is completely soluble in dilute acid, and will give him all the information he requires in the separation of the groups of proteins we have mentioned. The exact way in which this information is to be used for this purpose can probably be devised by the reader himself. In any case, it is fully described in works devoted to practical physiological chemistry. Besides giving our summary in this semi-diagramatic form we shall mention that the only methodical way in which a qualitative analysis of any kind can be carried out consists in noting down at each stage every substance which may possibly be present in each filtrate or precipitate; then, as the analysis proceeds, one or more of these possible constitutents become eliminated and struck off from the list at each step until only those remain which are actually present. We need hardly remind the student that he can at any stage determine whether he has already exhausted all the proteins of his solution by trying some of the protein colour reactions.

Summary.

THE SOLUBILITIES OF THE CHIEF CLASSES OF PROTEINS.

		Distilled water	Dilute salt solutions	Dilute acids	Dilute alkalies	Half-saturated ammonium sulphate	Fully-saturated ammonium sulphate
Typical native proteins, coagulated on heating	Albumins	—	—	—	—	—	
	Globulins		—	—	—		
Products of Peptic digestion	Metaproteins			—	—		
	Albumoses (1)	—	—	—	—		
	(2)	—	—	—	—	—	
	Peptones	—	—	—	—	—	—
Conjugated Proteins	Mucin				—		
	Casein				—		
	Nucleoproteins				—		
	Gelatine	—	—	—	—		

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A very readable account of the properties of the individual groups of proteins is to be found in

Practical Organic and Bio-Chemistry, by R. H. A. PLIMMER. (London: Longmans, Green & Co.)

A scheme for the separation of the various proteins is given in the *Practical Physiological Chemistry* by Cole, already referred to at the end of Chapter I.

CHAPTER III.

THE DIGESTION OF PROTEINS.

"Damit ein Heiligthum aufgerichtet werden kann, muss ein Heiligthum zerbrochen werden."—*Nietzsche.*

So far we have been considering the chemical constitution and properties of the proteins; we have studied their behaviour in test-tubes. But our ultimate object is to investigate the changes which these substances undergo in living tissues; to study the uses to which they are put in the body. Now before any foodstuff is utilised by the body, whether it be a fat, a carbohydrate, or a protein, it is, in general, changed and decomposed during the chemical processes of digestion. The materials with which the tissues have to work are therefore not the unaltered constituents of the diet, but the products arising from these when they are submitted to the action of the powerful digestive juices. In order, then, to understand the behaviour of proteins in the tissues we must first enquire into the nature of the changes which these substances undergo during digestion, and of the simpler compounds which are thereby produced.

This will be to some extent a familiar story. For example, it is a matter of common knowledge that the enzyme which commences the attack on the proteins of the food is the **pepsin** of the gastric juice. In the acid medium furnished by the hydrochloric acid, also secreted by the stomach, this enzyme breaks down the complex proteins of the food—such as the albumins

and globulins—into the simpler non-coagulable proteins, such as metaproteins, albumoses and peptones. Leaving the stomach, the food mass, now called chyme, passes into the duodenum, and is there acted upon by a second proteolytic enzyme called **trypsin.** This enzyme is ultimately derived from the pancreas, but is not present as such in the pancreatic juice. This secretion contains the enzyme only in the form of an inactive precursor known as trypsinogen, which itself has no action on proteins, but which is converted into active trypsin when it meets a substance known as **enterokinase,** which is secreted into the intestinal juice by the mucous membrane of the small intestine. This enterokinase is an example of the group of substances termed "kinases," which have the power of activating the mother substances or precursors of enzymes. We shall not now discuss the mechanism of this activation by means of kinases, but shall reserve the topic for a later chapter devoted to the study of the characteristics of enzymes (p. 176). But we might point out in passing that there would seem to be an important advantage in having the trypsin secreted in this masked form. For the pancreatic juice, as we shall again mention later, contains other enzymes—a lipase acting on fats, and an amylase acting on starch; and further, enzymes in general, and this lipase and amylase in particular, seem to be proteins, so that the result would be that if they were present in the same solution, together with the powerful proteolytic enzyme trypsin in an active form, they would be digested away and lost. As the trypsin cannot become active until it is actually in the intestine, such an undesirable result is avoided. Doubtless also this arrangement serves to protect the pancreatic tissue itself from being attacked

by the powerful enzyme whose precursor it produces. Trypsin differs from pepsin in acting in an alkaline medium, for the bile, pancreatic and intestinal juices which are poured simultaneously into the duodenum under the chemical stimulus evoked by the acid chyme, together contain enough sodium carbonate not only to neutralise the hydrochloric acid which has come over from the stomach, but further, to render the whole food mass alkaline. The action of trypsin on proteins is fundamentally similar to that of pepsin, but the hydrolysis of proteins brought about by trypsin is more complete than that brought about by the gastric enzyme, for instead of stopping at the albumose or peptone stage, it continues until the individual amino-acids are liberated. Nevertheless, there are some linkages in the molecules of native proteins which are more easily attached by pepsin than by trypsin, and in many cases it has been found that the most rapid method of breaking down a protein is to submit it first to the action of pepsin, which hydrolyses it to the peptone stage, and then to continue the hydrolysis to amino-acids by means of trypsin.

There is a third enzyme which is concerned with the digestion of proteins: this is the **erepsin,** which is a constituent of the intestinal juice. This enzyme has no action on native proteins, but it has a powerful hydrolytic action on the products of their partial breakdown such as peptones and albumoses; it serves therefore, to separate any amino-acids which may happen to have escaped the action of trypsin and so to have remained linked together. It is interesting to note, however, that erepsin can hydrolyse casein, the main protein constituent of the food of an infant.

It is important to realise that the breakdown of proteins

in the alimentary canal is a change similar to that which is brought about by boiling up these substances with dilute acid. In both cases the protein undergoes a process of hydrolysis as described on page 15, but in the presence of the digestive enzyme the breakdown occurs at a much lower temperature than is required in the presence of the acid only. The process of digestion, then, is one of hydrolysis, and we have come to the conclusion that in the digestion of proteins this hydrolysis goes so far as to liberate the various amino-acids which make up the protein molecule. This view has been based on the observation that the ferments of the alimentary canal are capable of bringing about so complete a change when examined *in vitro*, but it is important to add further arguments in support of the belief that such a change actually occurs in the body, because this view has but comparatively recently become generally accepted.

It used to be thought that so long as the digestive process converted the food into soluble products whose molecules were sufficiently small to permit of their diffusion through the mucous membrane of the alimentary canal into the blood, the whole object, if we may so express it, of digestion had been accomplished. Now peptones, it will be remembered, are particularly distinguished for their easy solubility, and they will diffuse, albeit slowly, through membranes. They would seem; therefore, to answer the criterion of fully digested proteins. But this is the old view. We have now very many reasons for believing that the digestion of proteins does not stop at the peptone stage, but continues until the free amino-acids are liberated.

Firstly—not only can we show that the proteolytic enzymes of the alimentary canal are *capable* of breaking

down the proteins to the amino-acids, but we can also show that they exercise their capability in the body. For if the proteins are broken down to amino-acids, and are absorbed as such, then we should be able to detect the amino-acids in the blood leaving the alimentary canal in the portal vessels while absorption of proteins is going on. It might be thought, at first sight, that this would be quite an easy thing to do, and that it would be necessary only to analyse the blood leaving the alimentary canal in order to find out whether there are any amino-acids in it. But it is not really quite so easy as this, because the blood always contains a certain quantity of amino-acids, so that all we could hope to find would be an increase in the amount of amino-acids contained in the blood flowing through the portal system. Further, this increase will not necessarily be a large one, as the blood-flow through the gut is so rapid that quite a large amount of amino-acid might be carried away from it with only a small increase of concentration of amino-acids in the blood. Each cubic centimetre of blood does not carry much amino-acid, but a large amount of amino-acid is carried because the number of cubic centimetres of blood flowing per minute is large. So that what we have to do is to detect a small increase in the concentration of the amino-acids in a fluid where there is a considerable quantity of these substances already present. Our task thus resolves itself into a quantitative determination which, as a matter of fact, can be carried out by taking advantage of the general reaction of nitrous acid with amino-compounds, to which we have already referred (p. 11). In other words, we can estimate the total amount of amino-acid in blood after suitable removal of the proteins by observing the volume of nitrogen which is evolved

when the filtrate is treated with nitrous acid. But in order to identify the particular amino-acids which are

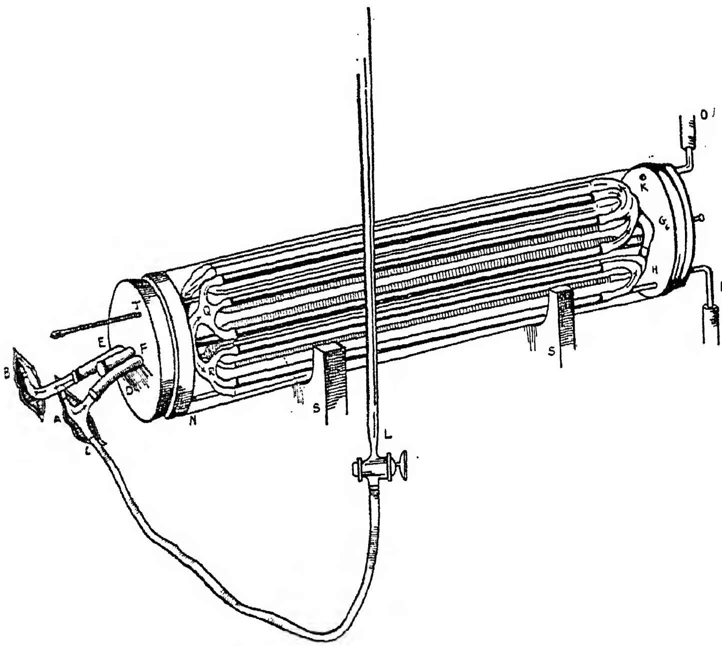

FIG. 1. The Vividiffusion Apparatus (after Abel).

The system of collodion dialysing tubes is seen fitted into an outer glass barrel filled with Ringer's solution. The blood which circulates through these tubes is drawn from the animal by means of a cannula inserted at A, and is returned through the cannula B. The burette L contains hirudin (leech-extract), which is run into the blood in small quantities to prevent clotting.

being absorbed it has been found possible to recover them from the circulating blood. This can be done by allowing the blood to pass through a long collodion tube surrounded by a bath of Ringer's solution. As the blood goes through,

the amino-acids, among other things, diffuse out, and the rate at which they diffuse out depends on their concentration in the blood. The blood is returned to the circulation after having traversed the length of the collodion tube. This is called the **vividiffusion** experiment, because we are allowing substances to diffuse out of the blood of a living animal (see Fig. 1). It has been found that if the experiment is performed on an animal which is digesting a good meal of proteins the amount of amino-acid that accumulates in the Ringer's solution in a given time is much greater than that which would be obtained from a starving animal under otherwise similar conditions. This shows that the form in which digested proteins are absorbed from the alimentary canal is that of free amino-acids. And further, it is possible to recognise in this dialysate of the portal blood many of the individual amino-acids with which we are already familiar as products of the complete hydrolysis of proteins outside the body.

Now, secondly, if the proteins of the food are completely broken down to amino-acids during digestion, we should be able to replace all the proteins of an animal's diet by the appropriate mixture of amino-acids without impairing its nutritional condition. This experiment has been tried by Abderhalden on a dog, which achieved fame for itself by consenting to eat the amino-acids from its master's laboratory shelves in place of its normal diet of juicy meat. It was found that the animal could easily obtain the nitrogen it required, once it had been induced to eat sufficient of the artificial food mixture which contained no proteins, but only the requisite proportions of tyrosine, tryptophane, histidine, glycine and the other amino-acids. In another experiment the dog actually put on flesh during a period of several months while it was

receiving only the amino-acid mixture obtained by the complete hydrolysis of meat.

This is a convenient point at which to mention that there is a very good reason for believing that in any case peptones are not absorbed in any quantity from the alimentary canal. The reason is this—that the peptones, if they get into the blood stream, are poisonous; they produce a very harmful fall of blood pressure, they destroy the normal coagulability of the blood, and they upset the permeability of the walls of the blood capillaries, so that increased lymph is produced, and symptoms resembling those of nettle-rash, result. Furthermore, the peptone appears in the urine, giving rise to the condition of peptonuria. As these effects do not follow the taking of a heavy protein meal we conclude that peptones do *not* represent the form in which the digested proteins are carried in the blood stream.

When biochemists first put forward the idea that the proteins were completely digested to amino-acids, it was at once objected that if the complex proteins are broken down to this extent, then a large portion of the chemical energy of the protein of a meal must be lost during the process; but actually this is found not to be the case. Hydrolysis is a chemical change which involves very little change of total energy in the reacting substances, and it is a fact that if we take a given weight of a protein and burn it up in the bomb calorimeter, then take the same weight of the same protein, hydrolyse it into its constituent amino-acids, and then burn these in the calorimeter, we obtain almost as much heat from the combustion of the amino-acids as we do from the original protein. So that this process of protein digestion is not wasteful of the chemical energy of the food.

Lastly, we can see a very definite biological significance in this complete breakdown of the food proteins. Not only is it necessary that these should be converted into soluble and diffusible substances which can be transported to the tissues, but these products of digestion must be such as can be utilised by the tissues, that is, they must be **assimilable.** Now it is not very surprising that, in order to convert the complex proteins of the food—the sheep's proteins which we eat as mutton, for example—into the equally complex but widely different proteins which make up human tissues, it should be necessary to break down the food proteins into their constituent units, and from these to build up the elaborate structures of those protein molecules which form the material basis of our own life processes.

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In these works are given the references to the papers where Abderhalden's feeding experiments with amino-acids are described. In particular, the student should refer to the article entitled

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Abel's account of the vividiffusion apparatus is contained in an article:

On the Removal of Diffusible Substances from the Circulating Blood of Living Animals by Dialysis. (*Journ. of Pharmacol. and Exptl. Ther.*, Vol. V. (1914), p. 275.

Lastly, Van Slyke's method for the determination of amino-groups is described in two papers entitled:

The Quantitative Determination of Aliphatic Amino Groups. (*Journ. of Biol. Chem.*, Vol. IX. (1911), p. 185, and Vol. XII. (1912), p. 275.

CHAPTER IV.

THE METABOLISM OF PROTEINS: THE USE OF AMINO-ACIDS AS FUEL.

"Erscheinungen, die sich auf chemische Veränderungen, sowohl der Bestandteile der Zelle sellst, als des umgebenden Cytoblastems beziehn; diese kann man metabolische Erscheinungen nennen."—*Schwann.*

We are now in a position to consider the question of the metabolism of proteins. The breaking down of the proteins of the food into their constituent amino-acids during digestion is not a process which, strictly speaking, is included among metabolic changes, for it occurs in the cavity of the alimentary canal, which is a space morphologically external to the actual tissues of the body. The term metabolism is reserved for such chemical changes as take place in the living cells themselves. During digestion the proteins, and indeed the foodstuffs in general, are merely prepared for the metabolic changes which they must undergo. It is only when the amino-acids have been absorbed from the digestive tract that their metabolism begins. Looking ahead, for a moment, we shall see that the end products of metabolism appear in the urine, for it is in the urine that most of the waste products produced during the chemical changes in the body are got rid of—with the exception, of course, of carbon dioxide and water vapour, which pass off from the lungs, and also of such substances as iron and calcium, which are excreted by the large intestine. So that our problem is to trace the whole range of chemical changes which intervene between the absorption of the amino-acids and the formation of

the constituents of the urine. Not that we can hope to deal with such a subject completely, for some of the stages in the chemical change lead to the building up of living protoplasm, about whose constitution we really know very little; but, nevertheless, the general principles of the subject have been made out. Incidentally one ought to mention that it is because the urine contains all the end products of metabolism that the analysis of this body fluid occupies such an important position in bio-chemistry; for not only is it in the urine that the products of normal metabolism are accumulated, but here also are to be sought the abnormal products of disordered metabolism which are indicative of disease.

To realise what is involved in this study of the metabolism of proteins we must think for a moment of the uses to which food is put in the body. Under ordinary circumstances the bulk of the food we eat is used as a fuel. It comes to be burnt up in order to provide the body with the energy of muscular movement, and with the heat required to keep it warm. But there is a second and smaller portion of the food which is used for repairing the wear and tear of the tissues of the body. Leaving aside, for a moment, the question of the material required for growth, and considering only the fully-grown organism, we find that fragments of its tissues are being continuously broken down and lost. For protoplasm is a very unstable complex, the continued existence of which depends upon the fact that normally it is built up at one place, so to speak, as fast as it is breaking down at another. The body is thus like any other machine—not only must it be supplied with fuel if it is to run, but also it must be supplied with small quantities of material in the form of spare parts for the

replacement of such components of its mechanism as become damaged or worn out during use. These two uses are subserved by the material taken into the body as food. Under ordinary circumstances most of this is used for fuel, but a certain proportion is required for the very important function of repairing the wear and tear of the tissues.

Now it is easy to see that in these processes proteins play a unique part. For, seeing that the tissues of the body consist so largely of proteins, it is obvious that it is only the protein part of the food which can supply that material which is required for the building up of the wear and tear, whereas all classes of foodstuffs, including fats and carbohydrates as well as proteins, can be used as fuel. Fats and carbohydrates cannot build up the tissues simply because these foodstuffs contain no nitrogen.

And not only are the proteins unique in this respect, but they have also another important use in the body, inasmuch as they furnish ready-made the ring groupings which the body is unable to synthesise, but which are indispensable for the manufacture of the nitrogenous constituents of essential internal secretions, enzymes and so on.

We shall commence our study of the uses of proteins in the body by considering the nature of the breakdown processes occurring in tissues; we shall then proceed to show how the lost constituents of tissues are replaced from the amino-acids absorbed from the alimentary canal; and, lastly, we shall describe the chemical changes suffered by the waste amino-acids of the tissues and the superfluous amino-acids of the food during their utilisation as fuel in the body.

To begin, then, with the use of the proteins—or rather

the amino-acids obtained from them—in the building up of the constituents of tissues. There are, of course, some cases in which an abundant supply of amino-acids is obviously required for the formation of new protein—for example, in the growing organism whose total amount of tissue protein is increasing. Then again, the convalescent requires amino-acids in order to rebuild the tissue proteins which were broken down during his illness. But even in the normal adult organism a certain amount of protein breakdown is continuously occurring, so that a certain constant supply of amino-acid is required to replace this. In order to understand the processes which constitute this aspect of the metabolism of proteins it is necessary to obtain an idea as to what is involved in this breakdown. We have already seen that living matter is a very complex mixture of substances, the most characteristic of which are proteins. These are combined to form an unstable system—protoplasm—which, even in the absence of any apparent external stimulus, is always tending to disintegrate. It must, therefore, be continuously supplied with the materials requisite for rebuilding its own structure. At once the question suggests itself: "What is the mechanism, and what are the products of this never-ending breakdown of the tissue protein?" The answer to the question becomes obvious when it is realised that in the complicated system there are not only proteins, but also proteolytic enzymes. Now it will be seen that it will require a very nice balance of conditions so to adjust things, that the protoplasm shall not fall a prey to the protein-splitting enzymes which it contains. A small disturbance of that balance, such as, perhaps, an excessive production of acid as the result of over-activity, or of insufficient circulation, will alter the conditions so

much that the proteins of the living cell may succumb to the activities of its own proteolytic enzymes. This can be easily illustrated by keeping a piece of muscle under aseptic conditions in an incubator at body temperature for a few days. Although bacteria are excluded, the proteins of the muscle break down, under the influence of its own proteolytic enzymes, until, finally, there is left little more than a mixture of amino-acids. This process of spontaneous disintegration of an isolated tissue is termed **autolysis.** Bearing these facts in mind it is not surprising to find that the break-down changes resulting from wear and tear in a tissue also consist in a hydrolysis of its proteins to their constituent amino-acids. In fact, the main changes undergone by the tissue protein during the wear and tear are different only as regards situation or position, and not in kind, from those changes which occur in the alimentary canal during the digestion of food proteins.

In order to understand how the amino-acids which are thus lost from the protein molecules composing living protoplasm are replaced, we must trace the fate of those amino-acids which, as we saw in the preceding chapter, are absorbed from the alimentary canal during the digestion of protein food. In the blood stream they are carried to all the tissues of the body, and it is found that after the absorption of a protein meal each tissue loads itself up with a supply of amino-acids from the common stock in the blood. The percentage of these uncombined free amino-acids in the various tissues can be shown by the nitrous acid method (p. 35) of analysis to be notably increased at this time. This is expressed in the curves given in Fig. 2. It will be noticed that the increase of amino-acid concentration is greater in the liver than in

other organs—a fact whose significance we shall explain later. Once the tissues have loaded themselves with amino-acids in this way they begin, so to speak, to choose out those which they particularly require for building up into their own substance, or, as raw materials, in the production of the essential constituents of some secretion. The others they sooner or later reject, so that, as the

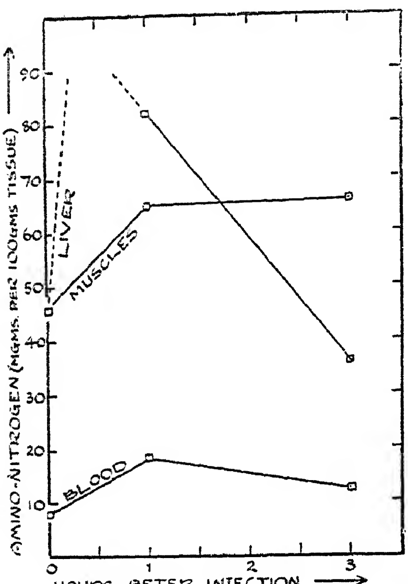


FIG. 2. Curves plotted from estimations by Van Slyke showing the extent to which amino-acids are taken up by various tissues of a fasting animal when these substances are injected into the blood stream.

The increased concentration of amino-acid nitrogen is most marked in the liver, but as the liver rapidly deaminates the amino-acids it receives, the exact maximum concentration in the organ is difficult to determine. On the other hand the amino-acids are retained by the muscles, and are used for the slow building up of their wasted substance.

curves shew, the percentage of amino-acid nitrogen in the tissues now begins to fall off. Thus, after an ordinary protein meal, the amino-acids are presented to the tissues, so that they may take their choice of those which they need. The rejected amino-acids represent that portion of the protein of the food which, not having been required for more important

uses, is to be used sooner or later as fuel. A certain fraction of these fuel amino-acids is turned loose again into the blood; the rest remain in the tissue. But wherever any particular amino-acid may be dealt with, the further treatment is the same for all amino-acids which are ultimately to be burnt. Those which are returned to the blood are carried round to the liver. This is how it happens that the liver accumulates amino-acids in such relatively large quantities while these are being absorbed—it receives all that the other organs have no use for.

Thus it comes about that of the amino-acids which are to be used as fuel some remain in the general tissues of the body, while the rest are concentrated in the liver. Now, in whichever position any particular amino-acid may be, the chemical changes which it undergoes during its utilisation for combustion are similar. Whether it takes place in the liver, or in the other tissues of the body, the first step in the use of an amino-acid as fuel consists in the removal of its nitrogen which, being non-combustible, is useless from the point of view of obtaining energy. **The nitrogen is eliminated by the removal of the amino-group as ammonia.** This process is termed **deamination.** The residual molecule now contains only carbon hydrogen and oxygen, and so is suitable for complete combustion. As a matter of fact, a partial oxidation occurs at the same time as the removal of the amino-group, in such a way that the non-nitrogenous residue from an amino-acid which has suffered deamination remains in the form of a **keto-acid,** that is, an acid in which the original position of the amino-group is now marked by the presence of the keto—or carbonyl group—CO. If we take as an example, alanine, amino-propionic acid, then

this on deamination gives ammonia and the particular keto-acid called **pyruvic,** thus:—

$$\underset{\text{alanine}}{CH_3.CHNH_2.COOH} + O \longrightarrow \underset{\text{pyruvic acid}}{CH_3.CO.COOH} + \underset{\text{ammonia}}{NH_3}$$

We shall have to deal in a moment with the further changes undergone by these keto-acids and the ammonia so produced. But before we do so it is important to emphasise the point that the process of deamination takes place both in the liver and also in the tissues generally, although perhaps on an ordinary diet it is in the liver that the greater part of the amino-acid suffers this change. It is easy to show this decomposition of amino-acids by direct experiment, for if we perfuse a surviving liver with a solution containing amino-acids we find in the out-flowing liquid products from which the amino-group has been removed. The keto-acid itself is difficult to detect under these conditions, as in contact with the liver tissue it readily undergoes further changes. This is the place at which to mention also that deamination is also the fate meted out to those amino-acids which have become split off from the molecules of tissue proteins during wear and tear. So that altogether we have undergoing deamination in the body the unwanted excess of amino-acid from the food proteins, and also those amino-acids which have become detached from the tissue proteins.

We can now return to a consideration of the further history of the ammonia and the keto-acids so formed. To consider that of the ammonia first.—This is liberated in tissues where there is an abundant supply of carbonic acid with which it combines, forming **ammonium car-**

bonate. The portion of this substance which is formed in the liver tissues then undergoes a process of dehydration in which two molecules of water are split off so that urea is formed:—

$$O{=}C\begin{array}{l}\diagup OH\\ \diagdown OH\end{array} \quad O{=}C\begin{array}{l}\diagup ONH_4\\ \diagdown ONH_4\end{array} \longrightarrow O{=}C\begin{array}{l}\diagup NH_2 + H_2O\\ \diagdown NH_2 + H_2O\end{array}$$

carbonic acid — ammonium carbonate — urea

As a matter of fact there is formed from the ammonia not only ammonium carbonate, but also some ammonium **carbamate,** which is a kind of half-way stage between the carbonate and urea.

This carbamate is also converted to urea by dehydration in the liver, only one molecule of water requiring to be removed in this case:—

$$O{=}C\begin{array}{l}\diagup ONH_4\\ \diagdown ONH_4\end{array} \quad O{=}C\begin{array}{l}\diagup ONH_4\\ \diagdown NH_2\end{array} \longrightarrow O{=}C\begin{array}{l}\diagup NH_2 + H_2O\\ \diagdown NH_2\end{array}$$

ammonium carbonate — ammonium carbamate

As a result then of the activity of the liver the alkaline ammonia is converted into urea,—a harmless soluble neutral substance which produces no ill effects while it circulates in the blood stream on its way to the kidney.

While we are discussing these changes we ought just to mention that the reverse reaction, namely, the formation of ammonium carbonate from urea, is well known. It occurs, for example, when urine is allowed to stand;

under the influence of a ferment **urease** contained in the bacteria which develop in the liquid the urea is converted into ammonium carbonate and the urine becomes ammoniacal and alkaline. This very reaction is also made use of in the most modern method for estimating the percentage of urea in urine and blood. In this case the necessary urease is obtained by making an extract of the soya bean, in which the enzyme is abundant. By means of this the urea is converted into carbon dioxide and ammonia, which latter is estimated by absorption in a measured volume of standard acid. Each molecule of urea gives two molecules of ammonia, so that from the quantity of ammonia formed the amount of urea can be readily calculated.

To return from this digression.—We have already emphasised the fact that at all events some of the amino-acids rejected by or split off from the tissues are deaminated where they are liberated without being first carried to the liver. As a result, ammonia is liberated in the tissues. But it is found that the ammonia so produced cannot be converted into urea in the general tissues of the body: for this purpose it must be conveyed to the liver, where alone the formation of urea occurs.

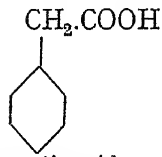
This ammonia, therefore, returns in the venous blood to the heart, and finally passes into the arterial system. Such of it as goes to the liver—and this will be a large proportion, because the blood supply to the liver is large—is converted into urea, while that which passes to the kidney is excreted unchanged into the urine. That the liver is the only organ in the body where ammonia is converted into urea is known from observations on animals in which the circulation through the liver has been short-circuited by joining the portal vein to the

posterior vena cava. Under these circumstances the blood supply to the liver is considerably reduced, and it is found that, as a result, the conversion of the waste nitrogen of the body into urea is much less complete than in a normal animal—a large proportion of it remaining as ammonia because the liver has far less opportunity of dealing with it. The accumulation of ammonia in this experiment shows that the other tissues of the body have not the power of converting ammonia into urea. An animal can be kept alive and in a healthy condition after such an operation has been performed on it, but only if it is given the very minimum of protein food. If it is given a large amount of protein food, then the ammonia liberated from that protein by the deamination which occurs in the tissues disturbs the normal reaction of the blood, so that the tissues in general become abnormally alkaline, and the animal goes into convulsions. The particular operation we have just mentioned is known as the establishment of an **Eck's fistula**; it is adopted instead of complete removal of the liver because it is much less drastic; among other things, it leaves intact the circulation to and from the alimentary canal.

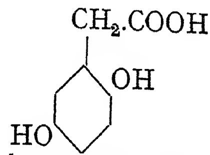
We turn now to consider the keto-acids which form the non-nitrogenous residues from the amino-acids after deamination. A very interesting way in which this formation of keto-acids has been proved is by taking advantage of a somewhat rare and apparently harmless disorder of metabolism known as **alcaptonuria.** In alcaptonuria, as the name indicates, the urine absorbs oxygen when it is exposed to the air, and turns black in colour. This behaviour is due to the presence in it of a substance called **homogentisic acid,** which is very easily oxidised in the air with the formation of dark coloured

products of oxidation. It will be seen that homogentisic acid is a di-hydroxy derivation of phenyl acetic acid:—

$CH_3.COOH$
acetic acid

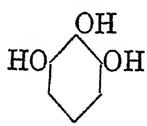


phenyl-acetic acid



homogentisic acid

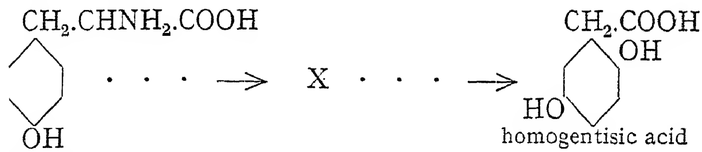
It is easy to understand why this substance should be so readily oxidisable. It will be observed that it contains two OH groups attached to a benzene ring: and all hydroxy-derivatives of this ring are readily oxidisable substances. The most familiar example of this fact is furnished by pyrogallol, which contains three hydroxy groups attached to the ring:—



This is so easily oxidisable that in alkaline solution it readily absorbs oxygen from the air forming dark-coloured products, and is therefore extensively employed as an absorbent for oxygen in gas analysis. It is interesting to note that homogentisic acid only absorbs oxygen when in alkaline solution, so that the urine of an alcaptonuric patient turns black on exposure to air most readily after it has stood for some time so that it has become alkaline by the bacterial formation of ammonia from the urea.

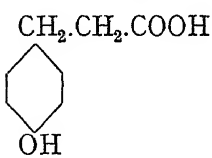
Now it is known for certain that homogentisic acid arises in the alcaptonuric patient from tyrosine, because if we withhold tyrosine from the diet the amount of homogentisic acid is reduced, and further, any tyrosine administered with the food gives rise to a corresponding

extra amount of homogentisic acid in the urine. We can say then that tyrosine in the alcaptonuric patient gives rise to homogentisic acid through certain intermediate stages thus:—

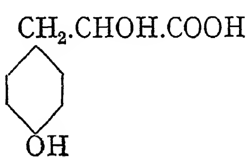


tyrosine homogentisic acid

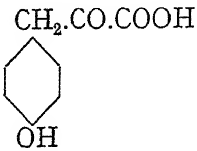
and we want to know what are these intermediate substances which we have called X. To do this we feed to the patient a few likely substances and see what happens. It is evident that if we hit upon a true intermediate stage between tyrosine and homogentisic acid then we should expect this to give rise to homogentisic acid just as tyrosine itself does. But if we choose a substance which does not occur on the line of metabolism, we should expect to find it excreted either unchanged, or in some form other than homogentisic acid. Actually we find that if we feed the saturated acid



corresponding to tyrosine, it does not cause an increased output of homogentisic acid in the urine; neither is the corresponding hydroxy-acid



converted to homogentisic acid during its passage through the body of an alcaptonuric; but if we give the corresponding keto-acid,



we find that it is quantitatively converted into homogentisic acid before it is excreted. So that we conclude that the keto-acid is an intermediate stage in the metabolism of tyrosine in the patient. It is supposed that in the normal body tyrosine similarly first gives rise through the keto-acid to homogentisic acid, but that here the change goes further, the homogentisic acid being oxidised right down to carbon dioxide and water. The difference between an alcaptonuric patient and a normal person seems to be that in the patient some enzyme is lacking which is necessary for breaking up this homogentisic acid.

The keto-acids are important substances because they represent, so to speak, the crossing point of two main lines of metabolism—that of carbohydrates and that of proteins. We have said that the proteins give us amino-acids. These on deamination give rise to keto-acids, and these are oxidised finally to carbon dioxide and water. Suppose, however, that the immediate oxidation of the keto-acids is not necessary for the body, then they are stored—and they are stored in the form of carbohydrate. The chains of the keto-acids are linked up to form glucose, and from this we readily obtain **glycogen** in the liver or other tissues. In the case of alanine the transformation is easy, for in its molecule alanine already contains a chain of three carbon atoms; we need to take only two such

molecules then, in order to give us the six-carbon chain of glucose. Of course, when the tissues require fuel for the production of heat and mechanical energy, this glycogen, just like the glycogen derived from other sources, is split down again into glucose, which is carried by the blood stream to any point where it may be needed.

It must be remembered that so far we have been dealing with the proteins which are to be used by the body as a fuel; and we have pointed out that the nitrogen of the amino-acid molecules is useless from the point of view of the obtaining of energy because it is not combustible. It is as useless as the ash in coal. Now it is found that if the body is going to use any amino-acid as a fuel it splits off the nitrogen from the amino-acid molecule as soon as this arrives at the living tissues, whether the amino-acid is to be burnt immediately, or whether it is to be stored until required. For if we measure the urea output in the urine during the period of **eight or nine hours** following a protein meal we find that during this time **an amount of nitrogen practically equal to that contained in the food is eliminated, and equally quickly whether the body is kept at rest or is employed on vigorous muscular work.**

This is a result of the most fundamental importance in the study of the metabolism of proteins. It was clearly realised by Voit as long ago as 1862 when he showed that during the day following the feeding of a kilogramme and a half of meat to a dog an amount of nitrogen exactly equal to that contained in the meat was eliminated from the body of the animal. Most of this excreted nitrogen was present in the urine; a little, however, was found in the fæces. On the other hand, during the same day there left the body as carbon dioxide and water, in urine, expired

air and fæces, smaller amounts of carbon and hydrogen than were present in the food. A certain portion of these energy-producing elements had evidently been retained in the body in the form of non-nitrogenous molecules.

The promptness with which the body deals with the nitrogen of a protein meal is illustrated by the curve shown in Fig. 3, which shows the hourly excretion of urea in the urine of a human subject after a single meal com-

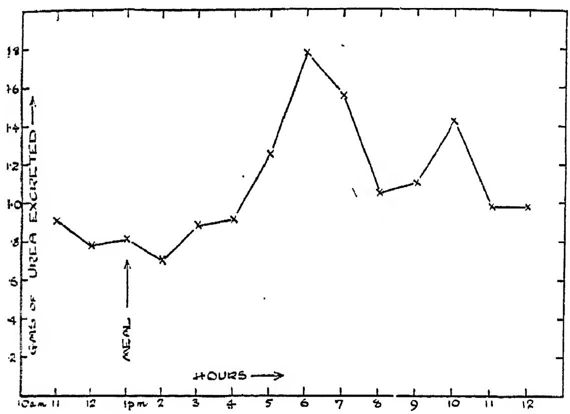


FIG. 3. The hourly excretion of nitrogen as urea in the urine during the period following a single protein meal.

To illustrate the promptness with which the body eliminates excess of nitrogen which is not required for repair of tissues (from Hopkins).

posed of 250 gms. of beef and 100 gms. of bread, following a fast of eighteen hours. It is seen that the urea excretion increases very soon after the taking of the meal, that it follows a variable course showing several maxima, and that it has returned almost to the fasting value about ten hours after the food was eaten. Naturally under more usual circumstances, when food is taken at frequent intervals, the increase in the rate of output of urea follow-

ing any one meal is masked to a great extent, because the nitrogen of the successive meals is eliminated in part simultaneously.

We have already compared the nitrogen of a fuel amino-acid with the non-combustible ash of coal. In order to pursue the analogy further let us take the case of a man who runs a steam engine. In the ordinary way the more he uses his engine the more coal ash he produces, and from the amount of ash produced in a day one could gather how much coal had been used. But the body does not work in the same way. It gets rid of the nitrogen at once on receiving the food, whether that is going to be burnt up now or several hours or even days ahead, so that the output of nitrogen depends only on the amount of protein food taken. The body behaves like a man who uses coal, but treats it chemically to remove the ash at once as soon as he receives it. The output of ash in this case would be determined entirely by the rate at which the coal was delivered to him, and would form no certain measure of the amount of coal actually being consumed. The reason for this behaviour is that the adult body has very little power of storing up excess amino-acids either unchanged or as new tissue protein; the carbon and hydrogen can ultimately form constituents of carbohydrate molecules and be stored in that form, but the nitrogen has to be lost. This nitrogen which is split off at once from the fuel amino-acids never forms a constituent of the living tissues of the body; the urea to which it gives rise is therefore said to be **exogenous** in origin; it has been produced *by* the tissues, but not from their substance. On the other hand, a certain amount of the urea of the urine will have been derived, as we have seen, from amino-acids which

have been liberated during the breakdown of the living material itself. This portion of the urea is referred to as **endogenous,** for it has been formed from the inner structure of the protoplasm itself.

This endogenous urea may be compared to the rust which is formed on the iron-work of a machine, and which, representing, as it does, a breakdown product of the actual fabric of the machine, must sooner or later be replaced if the machine is to maintain its working powers. An ordinary machine, however, differs from the body in the respect that while its breakdown product is non-combustible, the amino-acids which become split off from the body proteins can serve as fuel to the remaining tissues. It is the nitrogen of such amino-acids which goes to form the endogenous urea.

BIBLIOGRAPHY.

General accounts of the subject-matter of this Chapter are to be found in Cathcart's Monograph and Abderhalden's *Lehrbuch* referred to at the end of Chapter III. In addition, the student is recommended to read the article by Van Slyke entitled:

The Present Position of the Amino-Acids in Physiology and Pathology. (*Archives of Internal Medicine,* Vol. XIX. (1917), p. 56.)

And the series of papers by Van Slyke and Meyer on

The Fate of Protein Digestion Products in the Body. (*Journal of Biological Chemistry,* Vol. XII. (1912), p. 399, and Vol. XVI. (1913–14), pp. 187, 197, 213, 231.

For further information on Alcaptonuria, and similar metabolic disorders,

Inborn Errors of Metabolism, by A. E. Garrod. (Oxford Medical Publications, Oxford University Press.)

CHAPTER V.

THE METABOLISM OF PROTEINS (*contd.*): THE PRODUCTS OF TISSUE BREAKDOWN: CREATININE: NEUTRAL SULPHUR.

> "I would therefore call the protein metabolism which tends to be constant, *tissue* metabolism, or *endogenous* metabolism, and the other, the variable protein metabolism, I would call the *exogenous* or intermediate metabolism."—*Folin.*

IN the last chapter we saw that the chief nitrogenous product of the breakdown of tissue proteins is urea. But we found that urea is also a product of the utilisation as fuel of the excess amino-acids of the food. Now in any given case we cannot tell how much of the urea in a sample of urine is of exogenous and how much of endogenous origin, for the molecules of urea are not distinguishable according to their source. But there are substances which seem to be characteristic products of tissue breakdown, and which are not formed from the food. It will be realised that it is a very important matter indeed to obtain some kind of measure of the amount of endogenous metabolism going on in a subject—a patient for example—because this represents the rate at which the tissues are breaking down; and evidently one must know at what rate the tissues are breaking down if the patient is to be dieted suitably.

It is not difficult to understand the essential conditions which must apply to any substance whose amount in the urine shall serve as a measure of the total amount of tissue undergoing disintegration in the body. In the first place,

the required substance must be a product of tissue breakdown, and it must appear in the urine. Either it must be absent from the food, or any portion of it taken in by the mouth or formed from a food constituent must be destroyed before reaching the kidney. This will ensure that only the endogenous portion of this product, —only that which has been liberated from the tissues— shall appear in the urine. It is evident that any product which arises directly from the proteins of the food will be excreted in amounts which are proportional to the quantity of protein eaten, while any substance which is a product of the breakdown of tissue proteins will be excreted in more or less constant amount; for the rate of tissue breakdown will, on the whole, remain practically constant under normal physiological conditions. In order, therefore, to discover which particular constituent of the urine fulfils these conditions, it is necessary to vary the amount of protein in the diet of an individual, and to determine which substances he excretes in constant amount, in spite of the changes in his food. This has been done by Folin, and he has discovered that the chief urinary constituent which shews this constancy of output is **creatinine.** Indeed, the amount of this substance produced by a given individual is remarkably constant; the actual quantity being roughly proportional to the total amount of muscular tissue in the particular subject's body. For example, on a diet poor in proteins one of the persons investigated by Folin produced 1·6 grms. of creatinine per day, and on a diet rich in protein he gave out a practically unaltered quantity of this substance, namely, 1·55 grms. The contrast between this constancy of production of creatinine and the variability of the output of urea is remarkable. On the diet poor in protein the

same individual excreted 4·7 grms. of urea a day, while the amount was increased to 31·6 grms. when he received the richer food. These facts serve to mark off creatinine quite definitely as a truly endogenous product. And creatinine further obeys the other requirements of a product entirely derived from the tissues, for although when it is given by the mouth creatinine is excreted unchanged in the urine, that which appears under ordinary circumstances cannot be derived from the food, for it is not present in any ordinary articles of diet.

In order to understand the further relationships of this substance we must refer in outline to its chemistry. Creatinine is a ring compound whose constitution is most easily understood by considering that of related simpler substances. To begin with, we must remind the student that by replacing the divalent oxygen atom in the molecule of urea by the divalent imino-group $=NH$ we obtain a basic substance composed only of carbon, hydrogen and nitrogen, known as guanidine:—

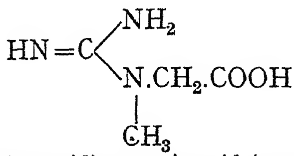
$$\underset{\text{urea}}{O{=}C\begin{array}{l} \diagup NH_2 \\ \diagdown NH_2 \end{array}} \qquad\qquad \underset{\text{guanidine}}{HN{=}C\begin{array}{l} \diagup NH_2 \\ \diagdown NH_2 \end{array}}$$

This guanidine readily enters into the composition of more complicated molecules, for example, guanidine-acetic acid is one of its more important derivatives:—

$$\underset{\text{guanidine-acetic acid}}{HN{=}C\begin{array}{l} \diagup NH_2 \\ \diagdown NH.CH_2.COOH \end{array}}$$

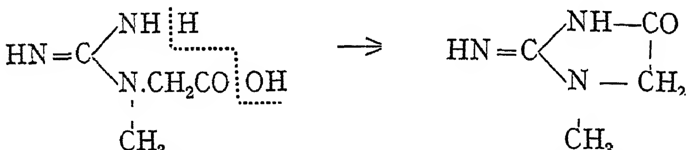
The substitution of a methyl group in place of the remaining hydrogen atom of the original NH_2 group

involved in the condensation gives us methyl-guanidine-acetic acid or creatine:—



methyl-guanidine-acetic acid (creatine)

a substance which we shall have to mention again later. Creatinine is the internal anhydride of this:—



creatine creatinine

One circumstance which renders creatinine so valuable as a measure of the rate of endogenous metabolism is the ease with which it is estimated. In order to determine the amount of creatinine in a sample of urine all that is necessary is to add some caustic soda and picric acid, when an orange-yellow colouration due to the formation of picramic acid is produced. The depth of this colour is compared with that given by a standard solution of creatinine, by means of a colorimeter; then, since the intensity of colour produced is proportional to the amount of creatinine, other things being equal, all the data are available for calculating the percentage of creatinine in the urine under examination.

The exact mode of origin of creatinine in the tissues is not yet certain. The muscles of the body contain very considerable quantities of the closely related substance **creatine**, to which we have already made reference and

which must be distinguished very carefully from creatinine itself. But although the two substances are so closely related chemically—creatine can be dehydrated to creatinine by boiling with a mineral acid—yet the extent to which the one is produced from the other in the body is not quite certain. The liver seems not to produce this change for the establishment of an Eck's fistula (p. 51), does not lead to a change in the quantity of creatinine excreted. It has been suggested that creatinine is formed at a constant rate in the body, and is converted into the creatine which is stored in the muscles. Then, when these are saturated with creatine, all further quantities of creatinine produced are excreted unchanged in the urine. On the other hand recently there has been a tendency to regard creatine as the primary product, and to suppose that the creatinine of the urine is formed from this.

Or it is possible that creatine and creatinine represent stages in entirely separate lines of metabolism which do not cross at any point, having no product in common, but which may be said to approach one another, as these two substances are closely related chemically. As far as living tissues are concerned, creatine and creatinine differ essentially from one another, in as much as creatinine is a ring compound while creatine is not. We have already mentioned the limitations of the power of the tissues to deal with ring compounds in discussing the amino-acids tyrosine (p. 53). It has been pointed out that the arrangement of carbon and nitrogen atoms in the ring structure of creatinine is the same as that in the iminazol ring, which, it will be remembered, occurs in the amino-acid histidine. Perhaps it is that, when histidine is liberated during the breakdown of a tissue protein, there is no enzyme present which is

able to split up the iminazol ring, so that this appears intact in the form of creatinine in the urine. Thus the creatinine excretion would be a measure of the amount of histidine liberated in tissues, and so of the rate at which the breakdown of tissue protein is occurring. If this be true, then, it follows that those quantities of the iminazol ring, which are taken in as histidine with the food, must suffer some other fate, and not become converted to creatinine. For, as we have seen, this substance is not excreted in increased amounts when excess of protein food is taken.

We have seen that the creatinine output of an individual is independent of the nature of his diet, because this substance is not a constituent of any foodstuff. There is another group of substances whose rate of excretion is not influenced by variations in the amount of protein eaten. These include the sulphur—containing amino-acid cystine (p. 17), together with certain of its decomposition products such as mercaptan (p. 16), in which the sulphur is in the form of—SH groups, and so is united chemically to hydrogen and not to oxygen as it is in sulphuric acid. This cystine and its decomposition products thus contain the **neutral sulphur** of the urine, as opposed, that is, to the acid sulphur of the sulphates. The reason why the output of neutral sulphur is independent of the amount of protein eaten is not that cystine is absent from the food, for this amino-acid is a common constituent of the protein molecule; it is due to the fact that the liver can oxidise cystine and convert its sulphur into inorganic sulphates, so that any portion of this substance which might be produced from the food protein, having necessarily to run the gauntlet of the liver before passing into the general circulation, will have no chance of

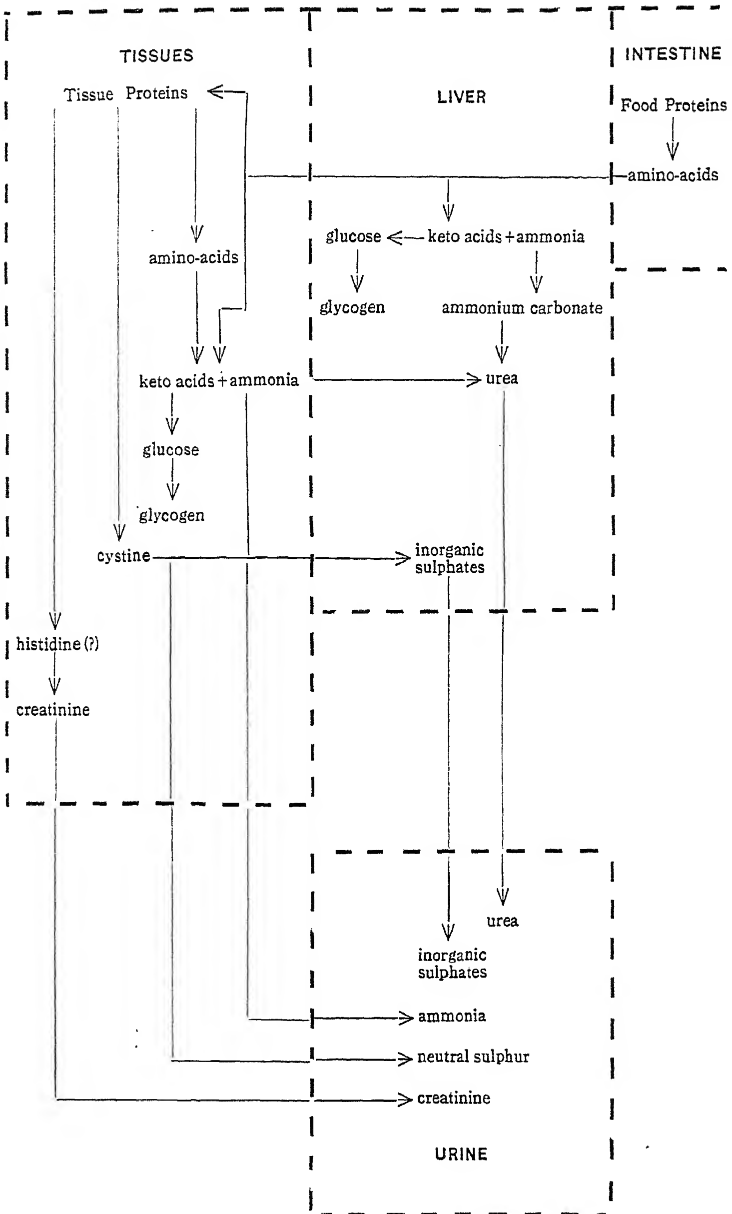
reaching the kidney unchanged. On the other hand, the tissues in general cannot completely oxidise the sulphur of any cystine liberated by the breakdown of their proteins, so that it is carried away in the blood stream. That portion of it which reaches the liver is oxidised, just as is that of the cystine brought in the portal blood from the alimentary canal, but the remainder will have a chance of being excreted in the urine from the blood which passes to the kidney before reaching the liver. Owing to the fact that the kidneys and liver are arranged in parallel with regard to the circulation, and not in series, a certain fraction of the total amount of the neutral sulphur liberated in the tissues will appear in the urine, a constant fraction determined by the relative rates of blood flow to kidney and liver respectively. Thus the amount of neutral sulphur in the urine is a constant fraction of the total amount produced in the body, and so is an indication of the rate of tissue breakdown.

From what we have said it would be expected that the neutral sulphur and the inorganic sulphate of the urine would show the same relationship to each other as we have found to exist between the creatinine output and that of urea: the former being formed from tissues at a constant rate, the latter varying in amount with every change in the food. And this indeed is actually the case. In the same metabolism experiment by Folin, as we quoted on page 60, it was found that on the rich protein diet the subject excreted 3·3 grms. of inorganic sulphate per day, while on the poorer food the amount fell to less than half a gram. On the other hand the daily excretion of neutral sulphur remained virtually constant throughout, being ·18 grms. on the former and ·20 grms. on the latter occasion.

Unfortunately, the amount of neutral sulphur present in the urine is not very easy to estimate. The difficulty is that the only way of estimating the neutral sulphur is to estimate the total sulphur of the urine, by oxidizing it all to sulphate, and also to estimate the already-formed sulphate of the urine. By subtraction of these results we arrive at the quantity of neutral sulphur, but the whole process involves two determinations, and so is not easy to carry out sufficiently quickly for (say) hospital purposes. Rapidity is a great asset in any biochemical method.

Summary of the Chief Chemical Changes which Proteins undergo in the Body.

The processes described in the last three chapters can be very conveniently summarised by means of the following diagram. It should be pointed out, however, that this diagram is merely one of several possible ways in which the outlines of the metabolism of proteins may be expressed; and that the student will only derive full benefit from his reading of this subject if he will work out his own schematic summary step by step as he follows the argument.


TISSUES
Tissue Proteins
amino-acids
keto acids + ammonia
glucose
glycogen
cystine
histidine (?)
creatinine
LIVER
glucose
keto acids + ammonia
glycogen
ammonium carbonate
urea
inorganic sulphates
INTESTINE
Food Proteins
amino-acids
urea
inorganic sulphates
ammonia
neutral sulphur
creatinine
URINE

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The fundamental paper by Folin on

A Theory of Protein Metabolism (*Amer. Journ. Physiol.*, Vol. XIII. (1905), p. 117),

should be read by all students of bio-chemistry.

With regard to creatine and creatinine—although we have dealt with them in barest outline as is appropriate in an introductory volume, yet there is an interesting and extensive literature dealing with the puzzling relationships of these substances. For general accounts, see:

Physiological Chemistry, by A. P. MATHEWS. 3rd edition, 1921. (London: Baillière, Tindall & Cox.)

The Metabolism of Creatine and Creatinine, (Myers and Fine, *Journ. Biol. Chem.*, Vol. XXI. (1915), p. 583.)

Zur Frage der Kreatinbildung in tierischen Organismus. (Riesser, *Zeitschr. f. Physiol. Chem.*, Vol. LXXXVI. (1913), p. 415.)

The Physiology of Creatine and Creatinine, (Hunter, *Physiological Reviews*, Vol. II. (1922), p. 586).

The view that creatine is formed from creatinine is put forward by Mellanby in

Creatin and Creatinin. (*Journ. Physiol.*, Vol. XXXVI. (1907–8), p. 447.)

The relation of creatinine to the maintenance of muscle tone is dealt with by Pekelharing and Harkink:

The Excretion of Creatinin in Man under the Influence of Muscular Tonus. (*Proc. Roy. Acad.*, Amsterdam, Vol. XIV. (1910), p. 310.)

Lastly, the importance of the parathyroids for the metabolism of guanidine derivatives is described in a series of papers in

Quart. Journ. Exptl. Physiol., Vol. X. (1916), by Noel Paton and Findlay,. and Burns and Sharpe.

CHAPTER VI.

THE NITROGEN EXCHANGES OF THE BODY AS A WHOLE: NITROGENOUS EQUILIBRIUM: PROTEIN STARVATION: THE USES OF PARTICULAR AMINO-ACIDS.

"Enough is as good as a feast."—*Old Proverb.*

In the preceding chapters we have considered the chemical properties of proteins and amino-acids, and the *details* of the changes which these undergo in the body. It is necessary for us now to study the metabolism of nitrogen in a more general way—fixing our attention on the uses of proteins in the body, and on the quantities of protein required by it under various conditions rather than on the details of the chemical changes to which these substances are submitted during their utilisation. We have dealt with some aspects of these subjects already; we have mentioned that the most important use of proteins is the replacement of the materials lost during the wear and tear of the tissues, and that any quantity of protein taken in excess of that required for this purpose is decomposed in such a way that its carbon and hydrogen are used ultimately as fuel while its nitrogen is got rid of—is wasted—in the urine. Now carbohydrates and fats can be equally well used as fuel in the tissues, but proteins alone can supply those amino-acids which are necessary for the upkeep of the living fabric. It becomes of interest, therefore, and of importance, to determine what is the minimum amount of nitrogen, in the form of protein, which must be present in an individual's diet in order that there shall be sufficient of this essential element

for the maintenance of his tissues, while at the same time there shall be as small an excess of protein as possible left over for the less essential function of providing fuel.

This is an enquiry which is important, apart from its value as a piece of scientific knowledge. It acquired considerable prominence during the war, when rationing schemes had to be devised in order to ensure an appropriate distribution of the diminishing supplies of nitrogenous food. Then also an answer to this question is important from a general sociological point of view. The nitrogenous foods—meat, for example—form the more expensive items of one's diet: it becomes, therefore, a very important thing to know whether the earnings of the poorer classes of any community are sufficient to enable them to buy at least the minimum amount of nitrogen, in the form of proteins, which they need in order to maintain their tissues. For if in any community it happens that the poorer classes cannot afford to buy such a sufficiency of nitrogenous food, then they will be living in a permanent condition of under-nutrition. As a result, the foundations of such a society are weakened, so that the whole structure becomes liable to collapse.

Now we can decide whether the tissues of a subject are wasting permanently or whether they are being repaired as fast as they break down by comparing the amount of nitrogen in his food with that which leaves his body in the urine and fæces. The difference between the amount of nitrogen taken in and that in the fæces represents the amount absorbed from the alimentary canal into the body. If no more nitrogen than this is excreted in the urine then the tissues cannot be losing permanently a nitrogen; but if more of this element is excreted than is absorbed, then evidently some of the excreted nitrogen

must have come from the tissues, and therefore the amount of living matter in the body is diminishing. When an individual is giving out in his urine an amount of nitrogen equal to that which he is absorbing he is said to be in **nitrogenous equilibrium:** when this condition is satisfied we know that all the nitrogen lost from his tissues is being exactly replaced; for seeing that his intake and output of nitrogen are exactly equal, the total amount of nitrogen in his body must be remaining constant. Naturally, although the nitrogen given out from the body under these circumstances is equal in amount to that which has just previously been absorbed, the actual nitrogen atoms concerned in the two cases are not entirely identical. For part of the excreted nitrogen will have come from the tissues and to replace this endogenous portion an exactly equal quantity will have been retained from the food; only the remainder of the food nitrogen will pass directly over into the urine. Of course a growing organism cannot be in nitrogenous equilibrium, for it gives out less nitrogen than it receives, retaining the rest in the form of the new tissues which it is building up. But the adult, fully-grown organism cannot retain more nitrogen than is required to maintain his already-existing tissues. He cannot produce entirely new tissue proteins merely by eating excess of protein food, neither can he store nitrogen in any other form (p. 57) as he can store carbon as fat and carbohydrates. It follows, therefore, that if a man is in nitrogenous equilibrium on a diet containing a certain amount of protein he will be necessarily in nitrogenous equilibrium on a diet containing more protein, for he will excrete all the nitrogen over and above the minimum amount which is necessary for the establishment of nitrogenous equilibrium. Naturally, also, a

starving man cannot be in nitrogenous equilibrium, nor can a man who is receiving no protein food, for in each of these the breakdown of the tissues still occurs, so that endogenous nitrogen appears in the urine, while no nitrogen is being taken in to replace what is being lost.

There have been a number of different estimates of the minimum quantity of protein food required per day by a normal man in order to maintain nitrogenous equilibrium. As time has gone on, the tendency has been to assess the amount at a smaller and smaller value. One of the most recent attempts to determine it was that in which a laboratory servant in the Nutrition Institute at Copenhagen was the victim. They fed this man for a period of several months on a diet composed only of potatoes and margarine flavoured with onion! His fuel requirements were adequately supplied by the fat and carbohydrate he received, and, as a matter of fact, the small percentage of protein present in the potatoes was found to be sufficient to maintain nitrogenous equilibrium. As a result of analysis it was found that the intake and output of nitrogen of this unfortunate fellow were only about five grams a day, but medical examination at the end of the experiment failed to discover any departure from a condition of robust health. Proteins, on an average, contain about one-sixth of their weight of nitrogen, so that the daily consumption of protein in this case was about thirty grams, i.e. just over an ounce—an amount considerably less than is included in an average diet, chiefly because potatoes and margarine do not appeal to our English palate so powerfully as meat and eggs. The protein portion of one's diet is in general the most appetising.

These five grams of nitrogen represent, then, the

amount which must be supplied to a normal healthy man per day in order that the loss of nitrogen from his tissues shall be compensated. If he receive less nitrogen than this, this loss nevertheless continues at an undiminished rate, so that his body wastes. If he receives no food at all—not even carbohydrate and fat—then the rate at which his tissues break down is much accelerated. For now the tissue proteins are called upon to supply the fuel material necessary for the carrying on of the more essential functions of the body such as the heart-beat and the respiratory movements. While the non-nitrogenous fuel reserves—the glycogen of the liver and muscles, and the fat of the fat depôts—last, the extent to which the tissue proteins are drawn upon is small. The nitrogen output in the urine during the first stages of starvation is comparatively small; it sinks below the normal value for the well-nourished animal because there is now no nitrogen from exogenous sources to be excreted. But as the fat and carbohydrate reserves tend to become depleted the extent to which the tissue proteins are drawn upon gradually increases. They are hydrolysed and deaminated, and the resulting keto-acids are ultimately used as fuel, while the nitrogen is excreted as urea in the urine. As a result of the increased use of the tissue proteins for fuel the nitrogen output is also increased. This increase of nitrogen excretion usually precedes the death of the animal by only a few days. The body has but little protein which it can really spare for use as a fuel. When this little is exhausted, the animal has no fuel of any kind available for carying on its life processes, and it dies. The lack of available protein is shown in this last stage of starvation by the rapid fall in nitrogen excretion. These facts are illustrated in the curve in

Fig. 4, which is taken from the results of an experiment by Falck on a lean dog.

During this utilisation of the tissue proteins for the supply of energy it must not be supposed that the various organs of the body contribute of their substance to equal extents. Indeed, it is found that the relative loss of weight which has been suffered by any particular organ by the time that an animal dies of starvation may be very different from that which has occurred in another. On the whole it is found that the least essential organs lose the greatest

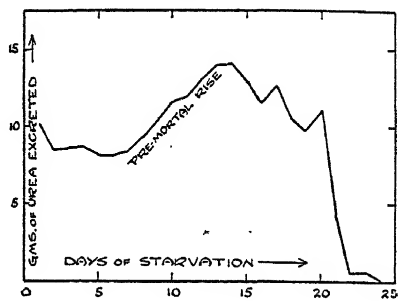

Fig. 4. The excretion of nitrogen as urea in the urine of a dog during three weeks' starvation.

At first, as a result of deprivation of protein food, the urea output falls to a low and more or less constant value. When the non-nitrogenous fuel reserves of the body are approaching exhaustion the animal begins to live at the expense of its own tissue proteins. This is marked by a rise in the amount of urea excreted, which reaches a maximum, and then falls off again when all the available protein has been utilised, and death is imminent. (Plotted from Falck's observations.)

percentage of their respective weights, while the substance of the more vital organs is much more carefully conserved. For example, while adipose tissue may have disappeared almost entirely from the body and the stores of glycogen in the liver have been so far exhausted as to have diminished the weight of that organ to half the normal value, while even voluntary muscles may have lost as much as a third of their original weight, the heart and brain will have given up a small fraction—a thirtieth or less—of their more valuable substance. There is much

significance in this behaviour of the body to starvation, for it is of the utmost importance to a starving animal that its heart-beat should be maintained at all costs in order that its brain may not be deprived of blood, so that the beast may remain alert and ready to take the fullest advantage of any opportunity that may offer of obtaining

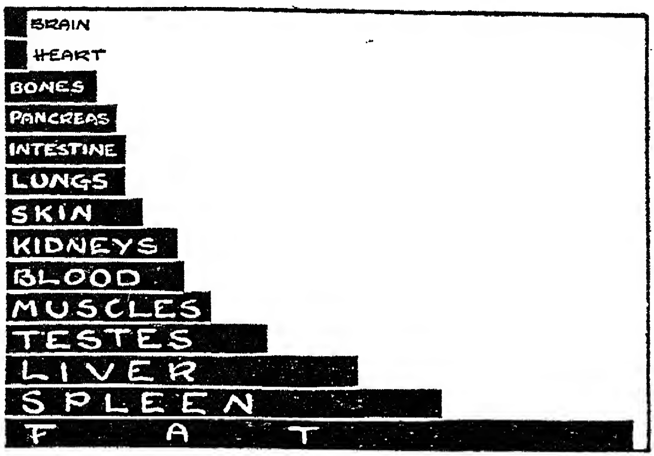

FIG. 5. A diagrammatic representation of the relative loss of weight of various organs during starvation as percentages of their initial weights.

Contrast the loss of 97 per cent. of the weight of the stored fat ot the body with the diminution of only 3 per cent. in the weight of the brain; and the loss of 31 per cent. of the weight of the skeletal muscles with a loss of only 3 per cent. of the weight of the heart. (Constructed from Voit's data.)

the food of which it stands in desperate need. The relative losses of weight of various organs during starvation are represented diagrammatically in Fig. 5.

So far we have spoken as if it were merely the total amount of protein nitrogen taken in per day which determined the condition of nitrogenous equilibrium. But further consideration will make it clear that the nature of the amino-acids in which the nitrogen is combined

is an important circumstance which bears on the question. For the amino-acids are not in general inter-convertible in the body. If any particular amino-acid is required for building up the tissues, then as a rule that particular amino-acid must be supplied ready formed in the food. If the tissue proteins are losing a certain amino-acid during their breakdown, such an amount of protein food must be supplied to the body as will contain sufficient of the particular amino-acid in question to replace that which is being lost, if nitrogenous equilibrium is to be maintained. For example, if histidine is being lost at a certain rate from the tissues, then for nitrogenous equilibrium the food proteins must contain sufficient histidine to replace this. Suppose, in addition, that the food proteins contain a large amount of some other amino-acid, say, glycine, which happens not to be required by the tissues, then the nitrogen of this excess of glycine must necessarily be wasted, since it is not in the required form. It follows, therefore, that a smaller amount of a protein which contains the various amino-acids in about the same proportions as those in which they are lost from the tissues, will be required for the maintenance of the nitrogenous equilibrium than of one in which the amino-acids occur in other proportions. We also see that the rate of nitrogen output in the urine of a man who is receiving just the minimum amount of protein sufficient for maintaining his tissues—it was about five grams in the case of the Copenhagen laboratory attendant—is not a true measure of the rate at which nitrogen is being lost from the tissues; for the nitrogen of the urine under these conditions represents not only that which has come from the tissues, but also that of the food amino-acids which the tissues had no use for, and which therefore were used merely

as fuel. This accounts for the observation that, in general, in order to maintain a man's tissues, it is not sufficient to supply him with the same amount of nitrogen in the food proteins as he excretes on a protein-free diet. It is necessary to supply a larger amount of protein than this, for usually a certain portion of some of the amino-acids will not be required by the tissues, and so will be useless for replacing their wear and tear.

These considerations supply the explanation for that prodigality of the body with regard to nitrogen which has long been realised. Without a knowledge of the needs of the tissues for particular amino-acids it would be surprising that even when the body is receiving less nitrogen than it requires for the maintenance of nitrogenous equilibrium, it does not retain all it receives, but excretes some in the urine. For if even a small amount of protein nitrogen be fed this leads to an increased output of nitrogenous substances even in a starving, wasting animal. If the amount of nitrogen in the diet is gradually increased, the difference between the amount given out and the amount taken in becomes smaller and smaller, so that the two curves representing nitrogen income and nitrogen output respectively gradually approach each other until, when there is a certain amount of nitrogen in the diet, they coincide. This is the point of nitrogenous equilibrium (see Fig. 6). Any further increase of food nitrogen beyond this point leads to an exactly equal output of nitrogen, and so to a maintenance of the equilibrium.

This discussion suggests one further topic with which we must deal briefly before leaving this subject of nitrogen metabolism, namely, the varying extents to which particular amino-acids are necessary to the body.

We know that tyrosine and tryptophane are absolutely essential for animal life; if these be omitted from an animal's diet it speedily dies. It has been suggested that these amino-acids are essential for the production of indispensable internal secretions, and it has been found that the active principle of the thyroid gland—

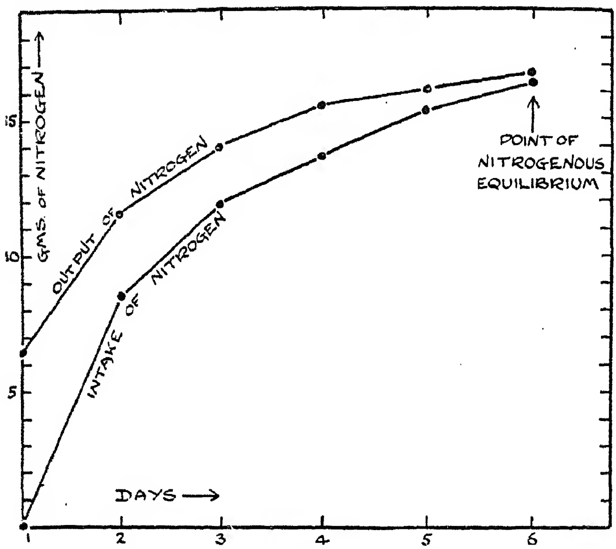

FIG. 6. Curves showing the gradual attainment of the condition of nitrogenous equilibrium on increasing the amount of protein fed to a dog.

Note that in order to attain the equilibrium it is necessary to give in the food a larger amount of nitrogen than is excreted during protein starvation because, in general, a portion of the food nitrogen will be in the form of amino-acids not required by the tissues. (Plotted from Voit's data.)

thyroxin—is an iodine derivative of indol, which is the ring structure present in tryptophane. Similarly, it seems likely that adrenalin is derived from tyrosine. In any case, the animal body cannot synthesise these essential amino-acids from other substances; they

must be consumed ready formed. It is for this reason that incomplete proteins such as gelatine, which contain no tyrosine or tryptophane, cannot be used as the sole proteins of a sufficient diet. On the other hand, plant protoplasm has the power of elaborating these compounds from their elements, so that it is from food plants that all the tyrosine and tryptophane in the whole animal kingdom has been derived. Plants can make ring compounds while animals cannot. Contrasted with these we have such simple amino-acids as glycine, which have been shown to be readily synthesised from other substances in the animal body, and so need not be given ready formed in the food. Occupying a position, as it were, intermediate between these two groups of the indispensable and the non-essential amino-acids, we have a group of amino-acids which, while not necessary for the well-being of an adult organism, must be present in the food of a young animal if growth is to take place. An example of this class of amino-acids is **lysine**—the α, ϵ di-amino derivative of caproic acid, which itself is the next fatty acid above valeric acid:—

$$\underset{\text{lysine}}{NH_2\overset{\epsilon}{C}H_2.CH_2.CH_2.CH_2.\overset{\alpha}{C}HNH_2.COOH}$$

Proteins are known which contain no lysine, and if these be fed to young rats the animals appear to remain quite healthy, but fail to grow up; they stay in a glorious condition of lasting youth, until growth is recommenced by the feeding of a normal full amino-acid diet. The amino-acids of the group to which lysine belongs seem not to be essential for the maintenance of the living structure when once it is fully formed, but they are necessary for building it up from the beginning.

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Hermann's Handbuch der Physiologie,

and in Tigerstedt's article in

Nagel's Handbuch der Physiologie.

In addition to Cathcart's monograph on Protein Metabolism, to which we have already referred, more recent works dealing with this subject are:

The Elements of the Science of Nutrition, by GRAHAM LUSK. 3rd edition, 1921. (Philadelphia and London: W. B. Saunders Co.)

Protein and Nutrition, by M. HINDHEDE. (London: Ewart, Seymour & Co. Ltd.)

Hindhede's classical article on protein minimum is entitled:

Studien über Eiweissminimum. (*Skandinavisches Archiv für Physiologie,* Vol. XXX. (1913), p. 96.)

Osborne and Mendel's articles on the importance of various amino-acids for growth are to be found in the *Journal of Biological Chemistry* from Vol. XII. (1912) on.

CHAPTER VII.

THE BIO-CHEMISTRY OF THE PURINES: NUCLEO-PROTEINS: URIC ACID.

"This racks the joints, this fires the veins."—*Gray.*

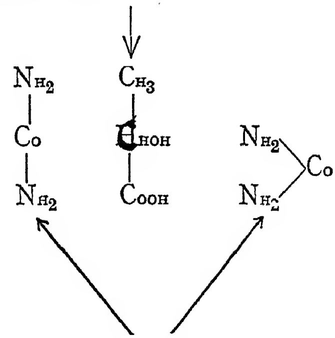
THERE remains yet a special group of proteins whose chemistry and metabolism we must consider before we shall have completed our study of nitrogenous substances. We refer to the **nucleo-proteins** which are characteristic constituents of the nuclei of cells. As we have already mentioned (p. 26) these are examples of conjugated proteins—substances whose complex molecules contain not only protein, but also non-protein groups. Without giving details at the moment, we will mention simply that the non-protein group of the nucleo-protein molecule contains, among other constituents, substances known as the **purine bases.** The importance of these bases from the physiological point of view is that they are closely related chemically to **uric acid,** and that during the course of metabolism they become converted into this substance. We may say, then, that just as urea and creatinine are characteristic products of the breakdown of ordinary proteins, so uric acid is the characteristic end-product of the metabolism of nucleo-proteins. Knowing this, the reader who has followed the argument contained in the previous chapters will have no difficulty

in appreciating the significance and importance of a study of the formation and output of uric acid from the body. For, apart from secondary factors, the amount of uric acid in the urine is a measure of the total amount of nucleo-protein suffering decomposition.

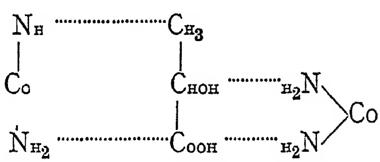
We will begin with a consideration of the chemistry of uric acid itself. This substance is a white crystalline solid which is but sparingly soluble in aqueous fluids. For example, it readily separates from the blood into the joints and tissues, so giving rise to arthritis and gout, and so constituting a valuable source of income to the medical profession. Uric acid is precipitated quantitatively from a solution made alkaline with ammonia on saturation with ammonium chloride; it can be estimated by titration with permanganate, also by various colourimetric methods. Its most characteristic reaction is known as the **murexide** test, which consists in the evaporation of a little of the material with strong nitric acid, and the addition of ammonia to the red solid residue. Under these circumstances a deep purple-red colouration is produced which resembles, superficially, the Tyrian purple obtained by the ancients from the sea-snail Murex.

Doubtless the reader will have already noticed in his reading the complicated ring structure of the formula of uric acid, and may have been struck by the apparent difficulty of remembering the arrangement of the atoms. But the difficulty vanishes if we remember that the chemist knows several methods by which he can synthesise uric acid in the laboratory, and that of these the easiest to understand is that in which he starts from two molecules of urea and a molecule of some acid containing a three-carbon chain—we will, as a matter of fact, take lactic acid as our example.

Here is the lactic acid:—

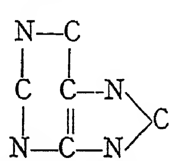


and here the two molecules of urea. Unimportant atoms are in small type. It will perhaps be objected that we have arranged these molecules in such a way as to suit our immediate purpose. Be that as it may—the point is that by appropriate means the chemist can induce these molecules to react in a way which can be represented like this:—



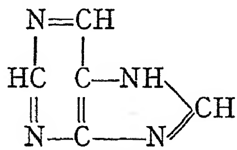
and so to give rise to a product containing the same double ring composed of nitrogen and carbon atoms which is characteristic of the molecule of uric acid, and from which uric acid itself can be readily obtained.

In this way it is easy to remember that the fundamental atoms in the molecule are arranged in a double ring structure thus:—



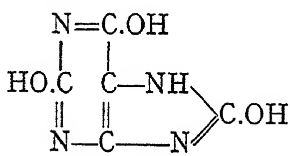
which is known as the **purine ring.** If we add hydrogen

atoms to complete the valencies we obtain the formula for a basic substance which contains only carbon, hydrogen and nitrogen, and is the substance **purine** itself:—



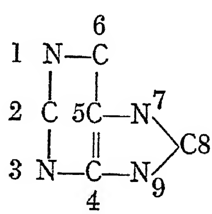
purine

Now uric acid is a tri-hydroxy-derivative of the purine ring in which the hydroxyl groups are all attached to carbon atoms thus:—



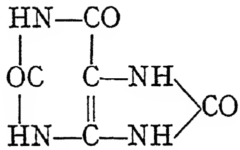
uric acid

As a matter of convenience, numbers are usually assigned to the atoms in the purine ring, and by convention it is agreed to label them in the following order:—



This numbering is purely arbitrary, but it is, of course, necessary to be able to refer to the positions of substituting groups in the molecule. Using this nomenclature we should say that uric acid is 2, 6, 8, tri-hydroxy-purine. As a matter of fact, one often sees its formula written in a slightly different way which expresses better

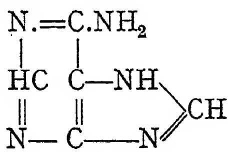
its relation to two molecules of urea and a three carbon chain:—



uric acid

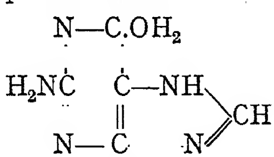
but this does not affect our present argument. Chemists believe that in this case the hydrogen atoms of the hydroxyl groups are capable of wandering from their appropriate oxygen atoms to the adjacent nitrogen atoms under some circumstances, so that it depends upon the conditions which formula the uric acid will obey. This behaviour is displayed by hydrogen atoms in some other substances; the chemists call it Tautomerism.

The other important purine derivatives with which we have to deal are the purine bases. These are amino-derivatives of the purine ring. Among them the most important are **adenine**, which is 6-amino-purine:—



adenine

and **guanine**, which contains a hydroxyl group as well as an amino-group and is 2 amino-6 oxy-purine:—



guanine

It is important to notice that the —NH_2 and —OH groups

in these substances are attached to carbon atoms which carry —OH groups in the uric acid molecule.

Adenine and guanine are the purine bases which occur most commonly in the non-protein groups of nucleo-proteins; it is most important to realise that **it is from these substances that the uric acid of the urine is derived** by a series of metabolic changes which we are about to study. Lastly, as a matter of interest, one might mention that the drug **caffein** is also a purine derivative—a methyl purine. Not that it enters into purine metabolism to any great extent, but as it occurs in such beverages as tea and coffee, and, furthermore, is a drug which finds use in physiological experiment and in medicine it is worthy of a passing notice.

Having dealt with the relationships of the chief purine substances which concern us, we are now equipped with the fundamental information which we need in order to understand the constitution of the nucleo-proteins. These form the essential constituents of nuclear matter, and seeing that the nucleus is in many ways the most complicated part of the cell, it is not surprising to find that its constituents are among the most complicated substances with which we have to deal.

As we have already mentioned, a nucleo-protein is what we term a conjugated protein—that is, that its molecule consists of a protein molecule combined with a group which is not a protein. In the particular case of the nucleo-proteins we have two protein molecules joined up to a non-protein group which is called **nucleic acid.** It is really like this: If we split off one protein molecule from the nucleo-protein we are left with a substance called nuclein; if we then split off a second protein molecule we

then have only our non-protein group, nucleic acid, remaining, thus:—

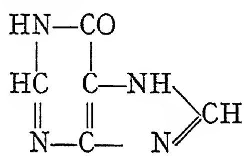
$$\begin{array}{c} \text{nucleo-protein} \\ \downarrow \\ \text{protein} + \text{nuclein} \\ \downarrow \\ \text{protein} + \text{nucleic acid} \end{array}$$

Nucleic acid itself is a very complicated substance. When it is decomposed it breaks up into phosphoric acid, purine bases and sugars. A group of one molecule each of phosphoric acid, purine base and sugar, is termed a **nucleotide,** and the molecule of nucleic acid is composed of a complex of such nucleotides just as the molecule of a protein consists of a complex of amino-acids. And just as we may have great variety among the amino-acids going to build up a protein molecule, so we may also find a considerable variety in the sugars or purine bases which go to form the nucleotides which compose the molecule of nucleic acid. As a result there is a great variety among the nucleic acids; but, no matter whether the nucleic acid is obtained from a plant like yeast, or whether it is isolated from human tissues, it is always found to consist of a complex of nucleotides, the essential structure of each of which is

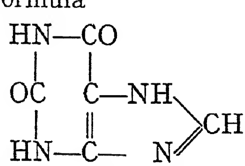
phosphoric acid—sugar—purine base.

We will begin the study of the changes which nucleo-proteins undergo in the body by considering the fate of those taken in with the food. The proteolytic enzymes of the alimentary canal split off the protein molecules, and from these form amino-acids in the ordinary way, leaving the nucleic acids free. These become further

broken down into their constituent nucleotides, and then each nucleotide is resolved into its phosphoric acid which goes to form the inorganic phosphates of the urine, its sugar which, when carried to the liver, may form glycogen, and its purine base, in which we are further interested. The purine bases are absorbed into the blood stream, and are carried to the liver and other tissues. The student who is familiar with the metabolism of proteins will feel no surprise at learning that the next process to which these amino-derivatives are subjected is one of deamination: the decomposition of the purine bases is begun by the splitting off of the amino-group. Now in the case of the amino-acids we found keto-acids and ammonia to be formed as a result of deamination. Exactly in the same kind of way we get keto-derivatives produced in the tissues by the deamination of adenine and guanine. In the substances so formed the original position of the amino-group is now marked by the CO group in the ring. Thus, the substance formed from adenine has the formula

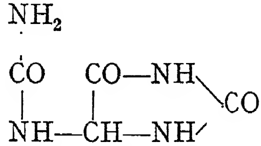


and is called **hypoxanthine,** while that formed from guanine has the formula



and is called **xanthine.** The conversion of xanthine (a di-oxy-derivative) and hypoxanthine (a mon-oxy-derivative)

into the tri-oxy-derivative uric acid requires a process of oxidation, and in most animals this seems to occur to a greater extent in the spleen than elsewhere. But in man this oxidation seems to take place in the tissues generally. Now while these changes have been suffered by the food proteins, the tissue nucleo-proteins have also been breaking down to some extent. Nucleic acids have been liberated. They have been split up into nucleotides, and these further into phosphoric acid, sugars and purine bases. These purine bases which are, of course, endogenous, become deaminated in the tissues themselves, and the oxidation of the xanthine and hypoxanthine so produced also takes place, so that leaving the tissues we shall have not only the uric acid which has been formed from food nucleo-proteins, but also any which has been formed by the breakdown of the nucleo-proteins of the tissues. In other words, we have leaving the tissues the uric acid both of exogenous and of endogenous origin. In most mammals the total uric acid so formed is not excreted unchanged in the urine, for most organs, but more particularly the liver, contain enzymes which bring about the further oxidation of uric acid to simpler substances. One of these is **Allantoin,** whose molecule contains the remains of a purine ring which has been, so to speak, burnt into and opened:—



allantoin

In the dog it seems that all the uric acid produced in the body is converted into allantoin; in other mammals it

seems as if a certain amount of the acid may be decomposed so completely that its nitrogen appears as urea. But while it thus comes about that in most mammals the greater bulk of the waste purine substances appear in the form of allantoin in the urine, in man the waste purins are present almost entirely as uric acid. Whether this uric acid in human urine represents the total amount of uric acid produced in the body is at present doubtful. Certainly uric acid administered by the mouth does not appear in the urine, but this may be because it is not absorbed from the alimentary canal. It is doubtful whether the human liver possesses that uricolytic power which we find in the case of the livers of other mammals.

By way of interest we might mention that the chief nitrogenous constituent of birds' urine is uric acid. This comes to be so because in the birds' liver the urea produced during the ordinary process of protein metabolism is actually built up into uric acid. Furthermore, in the bird this uric acid is built up from two molecules of urea and lactic acid: for on cutting out the liver from the circulation in a goose, for example, it is found that urea and ammonium lactate accumulate in its blood.

In the mammal also there is the possibility of a synthesis of uric acid, but from amino-acids and not from urea. It is found that if food containing nucleo-proteins or other purine substances is withheld from a man he continues to excrete a constant amount of uric acid per day, over a period which may last for months; but yet at the end of that time the man shows no evidence of abnormal loss of nucleo-proteins from his tissues, The conclusion is, then, that the organism has the power of synthesising the purine ring from some substances which

themselves do not contain it ready formed. Some evidence as to the raw materials used in this synthesis has been obtained by an experiment in which a number of rats were fed on food containing no arginine and no histidine. It was found then that the output of purines in the urine of the rats was very much reduced during the experimental

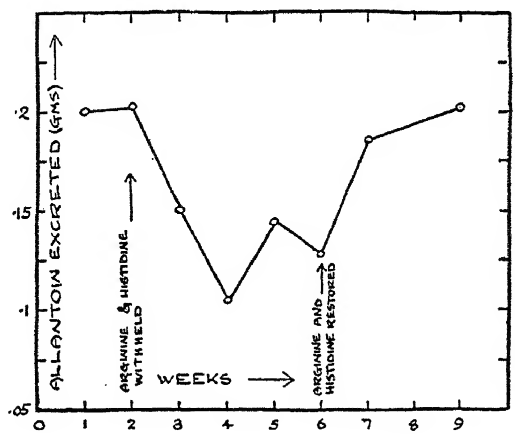
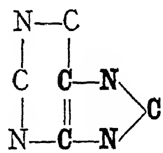


FIG. 7. The reduction of the output of the purine excretory product allantoin on withholding both arginine and histidine from the diet of a rat.

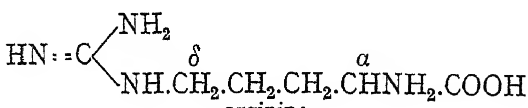
The allantoin excretion returns to normal when these amino-acids are once more added to the food. (Plotted from the results of an experiment by Hopkins.)

period, but returned again to normal when the full diet containing histidine and arginine was resumed (see Fig. 7). The omission of other amino-acids from the diet produced no such effect. It is not difficult to see how histidine and arginine might possible be the precursors of the purine ring in the body: Histidine, it will be remembered, contains an iminazol ring, and in one part of the purine

ring we have also that combination of carbon and nitrogen atoms which constitutes an iminazol ring, thus:—

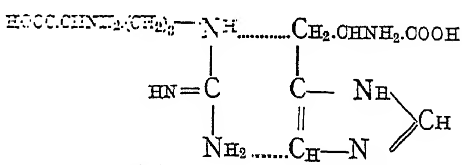


Arginine is an amino-acid which contains guanidine; it is in fact the α-amino, δ-guanidine derivative of valeric acid, the latter being the next acid in the fatty series above butyric:—



arginine

By re-writing the formulæ it is easy to see how arginine and histidine might conceivably be useful in building up the purine ring, thus:—

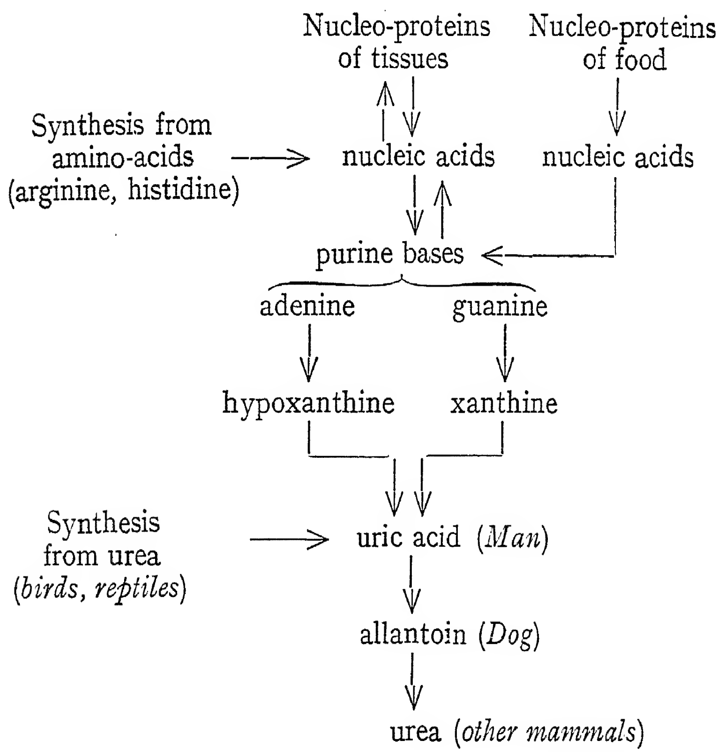


arginine histidine

It might be pointed out also that the same synthesis of the purine ring from non-purine substances can be observed during the incubation of an egg. For as the nuclei divide more and more nucleo-proteins are built up, so that the fully developed chick contains a much greater weight of purine derivatives than the original egg, although no food has been supplied from outside.

SUMMARY

We shall summarise in the following scheme our account of the chemical changes undergone by the chief purine derivatives in the body. But in doing so we shall refrain from mentioning specific organs or tissues where the changes occur, partly because they seem to occur to some extent in all tissues, and partly because the distribution of the ferments which influence these changes varies so widely from one animal to another.



BIBLIOGRAPHY.

The book most to be recommended to the student who wishes to study further the constitution and metabolism of the nucleo proteins is

Nucleic Acids, by W. JONES.

This is in the series of Monographs on Biochemistry.

Also in the same series is a volume entitled:

Oxidations and Reductions in the Animal Body, by H. D. DAKIN,

which contains a chapter on the production of uric acid. The synthesis of purine derivatives from amino-acids is dealt with by Ackroyd and Hopkins in an article entitled:

Feeding Experiments with Deficiencies in the Amino-Acid Supply. Arginine and Histidine as Possible Precursors of Purines. (*Biochemical Journal,* Vol. X. (1916), p. 551.)

CHAPTER VIII.

FATS AND THEIR METABOLISM: LECITHIN: CHOLESTEROL.

"Let me have men about me that are fat."—*Julius Cæsar.*

As usual in dealing with the bio-chemistry of any group of substances we will begin with a brief outline of the pure chemistry of the materials in question. From his previous reading of organic chemistry the student will remember that just as we can take an acid—acetic $CH_3.COOH$, for example—and neutralise it with caustic soda and obtain its sodium salt $CH_3.COONa$, so in an exactly similar manner we can cause the acid to react with an alcohol and obtain a salt of an organic radicle, the alcohol acting as a weak base. If we use ethyl alcohol, then acetic acid will give us ethyl acetate—$CH_3.COO\ C_2H_5$. Such a salt of an organic radicle is called an ester. One of the most characteristic reactions shewn by an ester is that which occurs when it is boiled up with caustic soda; the ester is thereby hydrolysed, its alcohol being liberated and its acid remaining as the sodium salt. Still taking ethyl acetate as our example, we can represent the process thus:

$$\begin{matrix} CH_3.COO\vdots C_2H_5 \\ Na\vdots OH \end{matrix} \longrightarrow \underset{\text{sodium acetate}}{CH_3.COONa} + \underset{\text{ethyl alcohol}}{C_2H_5.OH}$$

Now the fats are simply esters; they are salts formed by a union of an alcohol with acids. The alcohol which occurs in the fats is not so simple a substance as ethyl alcohol, but

is the somewhat more complicated one which is familiarly known as glycerine. Scientifically it is usually termed **glycerol,** and from its formula it will be seen that its molecule contains three—OH groups:—

$$\begin{array}{l} CH_2.OH \\ | \\ CH.OH \\ | \\ CH_2.OH \end{array}$$

There are three acids whose glycerol esters make up the common fats. The first is **stearic acid,** which is a typical member of the fatty series with a long straight chain, composed of eighteen carbon atoms. We write it for the sake of brevity thus:—$CH_3.(CH_2)_{16}.COOH$. The second acid which we find is **palmitic,** which is the next member but one below stearic and so contains a carbon chain two atoms shorter:—$CH_3.(CH_2)_{14}.COOH$. Lastly we have **oleic acid,** which has the same number of carbon atoms as stearic, but is unsaturated, a double bond occurring in the middle of the chain:—

$$CH_3.(CH_2)_7.CH{=}CH.(CH_2)_7.COOH$$

Seeing that the glycerol molecule contains three hydroxyl groups, it is evident that it will combine with three molecules of either of these acids. Now the ordinary fat of an animal does not consist of any single ester but of a mixture of the glycerol ester of stearic acid (often called tri-stearin) with that of palmitic acid (tri-palmitin), and of oleic acid (tri-olein). Tri-olein is a liquid substance, so that the more of this ester present in a fat the softer it is. Olive oil is practically entirely composed of tri-olein; pigs' fat (lard) contains a moderate proportion of olein while the hard mutton fat is practically devoid of it, its chief constituent being tri-stearin. Under normal circumstances the

stored fat of each animal is a definite mixture of these three esters, characteristic of its species, but, when excessive quantities of foreign fats are administered, these tend to be stored unchanged, so that the normal composition of the fat is departed from.

One of the typical points in the behaviour of unsaturated compounds is that they combine directly with halogens—iodine, for example. In particular, oleic acid and olein exhibit this property, so that the amount of iodine with which a fat will combine is a measure of the percentage of olein present in it. Hence the value of determining the "iodine number" in the investigation of fats. We have mentioned that if we take an ester and stew it up with caustic soda we get a sodium salt and the free alcohol. Exactly the same is true of the esters which make up fats: if these are acted upon by caustic soda, the alcohol glycerol is liberated and the sodium salts are formed of the fatty acids which are present. This process is called saponification—soap formation—because this is the process fundamental to the making of soap. Ordinary soap consists of the sodium salts of stearic and palmitic acids, and it is got by boiling up fats with caustic soda. Incidentally, of course, glycerol is liberated at the same time, and that is why glycerine is always produced at the soap works.

Let us now consider the sources whence the body obtains fats. The most direct source of supply is the fat which exists preformed in the food. Now although the body utilises the fat of the food as fat, and does not require to convert it into any other substance before it can store it or use it, yet the fat of the food is submitted to a very definite process of digestion before it is absorbed. This digestive process consists in the hydrolysis of the fat

into glycerol and fatty acids under the influence of fat-splitting enzymes, or **lipases.** A certain amount of such an enzyme occurs in the stomach, but it is uncertain whether or no this lipase is merely that contained in such portions of the duodenal contents as regurgitate through the pyloric sphincter; it is not certain that the stomach itself form the lipase. The chief lipase which occurs in the alimentary canal is that secreted in the pancreatic juice; it has received the name of **steapsin.** This exerts a very powerful hydrolytic action on fats, and the conditions of its action are favoured in a very marked degree by the properties of the bile which is poured into the duodenum simultaneously with the pancreatic juice. In the first place the bile is strongly alkaline, so that the pancreatic lipase acts in an alkaline medium. This means that the fatty acids, as soon as they are liberated by the action of the enzyme are converted into their sodium salts, or soaps. Now in an alkaline solution containing soaps it is found that fats possess the property of breaking up into very fine globules, and so of forming an emulsion in which the particles expose a very large total surface to the action of any enzyme which may be present. In the acid gastric juice free fatty acids are liberated, but no soaps are formed, so that emulsification does not take place, and no great amount of hydrolysis of the fat can occur on account of the smallness of the total surface it exposes to the enzyme. But when the fat passes from the stomach into the alkaline contents of the duodenum, that small amount of free fatty acid which it does contain becomes converted into soluble soaps, which, in dissolving, set up diffusion currents which break up the fat particles into the fine globules of an emulsion. Now the bile has the useful property of rendering this emulsion stable and permanent,

and of preventing the reunion of the separate globules. It possesses this power on account of the bile salts it contains. These are the sodium salts of complex organic acids. In order to understand the way in which they stabilise a suspension of fat globules it is necessary to consider the property of surface tension to a certain extent. It is a matter of every day observation that the surfaces of most liquids behave as if they were always tending to contract—as if they were enclosed, so to speak, in a kind of elastic skin. This behaviour gives rise to that form of energy that we call surface tension or surface energy. It is evident that the more tendency there is for the surface of a liquid to contract, the more difficult it is to increase that surface. Now the surface between the fat particles and water is of that kind which tends to contract; it has a high surface tension. In order to increase this surface then, it is necessary to add a considerable amount of energy to the system. In other words, the fat will not readily break up into small droplets, for this means an increase of total surface, but rather will tend to run together into one large mass of which the surface is a minimum. But the bile salts have the useful property of reducing the surface tension very considerably so that, once the fat has been broken up into globules, these have little tendency to run together again, and a permanent emulsion is formed. The fat is thus made to expose a large surface to the action of the enzyme, and so undergoes rapid hydrolysis. A further use of the bile salts is that they serve to keep in solution the otherwise insoluble fatty acids. So that even if the contents of the intestine become acid, the free fatty acids thereby liberated from their sodium salts are not precipitated if there be a sufficiency of bile present. It should be mentioned that any

fat which escapes hydrolysis in the duodenum is attached by a further lipase which is present in the intestinal juice.

We must now consider the mechanism by means of which these products of the hydrolysis of fats are absorbed from the alimentary canal. It will be remembered that the amino-acids resulting from the digestion of proteins are absorbed into the blood stream, through the capillary blood vessels of the villi of the small intestine; but the fats are absorbed by a different route. The mixture of glycerol and soaps passes into the columnar cells lining the villi, and there, in these cells, we find that the fatty acids and the glycerine which have been absorbed together are re-combined to form fat, which accumulates in the form of droplets. It thus appears that the only object, if we may so express it, in hydrolysing fat in the alimentary canal to fatty acid and glycerine, is to produce substances which are both sufficiently soluble to pass through the limiting membrane of the intestinal cell. If the cells of the intestine are examined histologically during the absorption of fat, it is found that as the fat globules pass from the edge of the cell that was facing the intestinal cavity they become larger and larger. Escaping from the opposite end of the cell, they find their way across to the central cavity of the villus which, on account of the presence of fat in the fluid it contains, is usually referred to as a central lacteal. This vessel is really a lymph channel, and its contained fluid—known as chyle—having become loaded up with fat globules, passes round in the lymphatic system and finally enters the blood by the thoracic duct. If the fat is not to be used at once for the purpose of obtaining energy it is stored unchanged in the fat depôts which are chiefly in the mesentery and the subcutaneous connective tissues.

Now we have already mentioned that the fat occurring in the fat depôts of an animal is, under normal circumstances, a definite mixture of stearin, palmitin and olein in fixed proportions, which are different for the various species of animals. It therefore follows that if an animal of one species is fed on the fat of a different species then there must occur a transformation of the mixture of esters present in the food fat into that different mixture of esters which constitutes the depôt fat characteristic of the animal which has received the food. This transformation occurs in the cells of the villi in which the fat is resynthesised during absorption, for the fat in the chyle is found to agree in composition with the depôt fat rather than with the food fat. But the power which the body possesses of bringing about this change is limited, for if a large excess of a foreign fat be fed, then some of this may be deposited unchanged in composition. Thus it is possible to replace the normal hard fat of a dog's body by a much softer fat of lower melting point by feeding it with large quantities of the semi-fluid pig's fat. This mixture of esters characteristic of a species may arise not only from the fat, but also from other constituents of the food—more particularly from the carbodydrates. The fattening of cattle on a diet containing abundance of carbohydrate is a matter of common experience; it has been a subject of exact enquiry in the case of the pig. Two pigs of the same litter, and of as nearly as possible equal weights, were taken; one was slaughtered at once, and the amount of fat in its body was estimated. It is assumed that the other similar pig started off with this same amount of fat. This second pig was then brought up on a carefully analysed diet for some months. At the end of this time it was found that there was much

more fat in its body than was present at the start, and that the increase was much more than could be accounted for by the amount of fat it had taken in with its food; therefore the carbohydrates of the diet must have given rise to fat. At one time it was thought that the proteins of the food could also give rise to fat in the body, because an animal on a lean meat diet most certainly puts on fat. But then later it was realised that the muscle fibres, of which lean meat is composed, contain a sufficiently large amount of fat to account for all that which is put on, so the proteins of the food do not give rise to new fat. This is a rather curious result, because we know that proteins will form carbohydrates, glycogen for instance, and so it might have been expected that the carbohydrates so formed might give rise to fats; but apparently it is not so. The explanation for this inability of the proteins to form fat is probably not that the requisite chemical stages are impossible in the body, but that the proteins stimulate the oxidation in the tissues to such an extent that no fat is left over to be stored; it is all burnt up at once (see p. 155).

The essential function of fats in the body is to act as fuel to the tissues. In this connection it should be pointed out that fat is a very economical form in which to store fuel. For the molecule of a fat consists almost entirely of carbon and hydrogen atoms. It possesses but few oxygen atoms—far less relatively than are present in a carbohydrate molecule, and no incombustible nitrogen such as is present in a protein molecule. Thus it comes about that, weight for weight, fat contains much more potential chemical energy than either carbohydrate or protein. A gram of fat on oxidation gives off 9·5 large calories, as compared with 5·6 calories obtainable

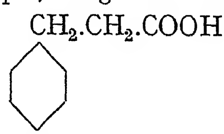
from a gram of protein, and only 4·2 calories from a gram of carbohydrate. The steps in the actual oxidation of fats are somewhat complicated. The fat in the fat depôts seems to undergo an elaborate process of preparation before it is carried to the tissues to be burnt. Seeing that when vigorous muscular exercise is taken at the expense of the stored fat of the body the liver becomes loaded up with fat, it is evident that it is in this organ that the preparatory processes mainly take place. These result in two chief changes. Firstly, the molecule is de-saturated so that double bonds appear in it. This renders it particularly liable to oxidation, for a double bond is a point of weakness in a molecule. Then also the fat comes to contain a large percentage of **lipoids**—substances such as **lecithin** and **cholesterol,** which are not true fats, but which seem to be intimately associated with the fats during their metabolism in the body. We shall refeı to these substances again in more detail in a moment. Meanwhile we have said enough to show the reader how it comes about that the fat which occurs in tissues—that is, that which is waiting in the protoplasm itself for oxidation—has a composition very different from that of the depôt fat of the animal.

With regard to the stages occurring during the actual oxidation of fats we have a certain amount of information, but there are many gaps in our knowledge of the subject. There is reason for believing that when a long chain such as occurs, say, in stearic acid, is oxidised in the tissues the carbon atoms are split off two by two from the end where the —COOH group is situated. The α-carbon atom and the —COOH group are oxidised to carbon dioxide and water, while what was originally the β-carbon atom of the long chain now becomes the centre of a new carboxyl

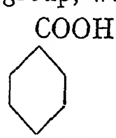
group. In this way the long carbon chain has been diminished by two carbon atoms and a lower member of the fatty acids remains for further similar oxidation. This change is usually referred to as β-**oxidation;** it may be represented as follows:—

$$CH_3.(CH_2).{_n}COOH \longrightarrow CH_3.(CH_2).{_{n-2}}COOH + CO_2 + H_2O$$

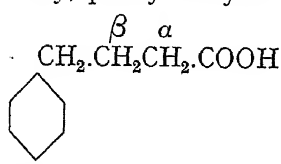
Evidence in favour of this view is obtained by feeding fatty acids into whose molecules a benzene ring has been inserted so as to prevent the final stages of the oxidation. Suppose, for example, we give an animal this substance:



—phenyl-propionic acid—then when two carbon atoms are removed from the side chain and the β-atom is oxidised to a carboxyl group, we obtain benzoic acid,



which cannot be further oxidised because the ring is stable. Suppose, however, we take the phenyl derivative of a fatty acid containing one more carbon atom in the molecule—say, phenyl-butyric:—



then we can oxidise away two carbon atoms as before and convert the β-atom into a —COOH group. But further than this the body cannot oxidise the molecule. The end stage in the oxidation is thus

$CH_2.COOH$

—phenyl-acetic acid. The reason seems to be that the carbon atoms can be oxidised away only in pairs: and that the removal of two carbon atoms from the molecule of phenyl-acetic acid would require the formation of a carboxyl group whose carbon atom was one of the six originally present in the ring. This would necessitate the opening of the ring—a change which the body is incapable of accomplishing. If the carbon atoms were removed one by one, however, there would seem no reason why phenyl-acetic acid should not become oxidised further to benzoic acid. Further, it is a significant fact that all the fatty acids that occur in nature have an even number of carbon atoms in their chains. The fatty acids with an odd number of carbon atoms in their chain can be synthesised chemically, but they do not occur naturally. It will be remembered that we said that stearic acid with eighteen carbon atoms, and palmitic with sixteen, occur in animal fats, but we did not mention the seventeen carbon acid because it is of interest only to the student of organic but not of bio-chemistry. Of course, this probably means that the fatty acids are not only broken down, but are also built up in the tissues two carbon atoms at a time.

And recent work has confirmed this idea. Without entering into details, we might mention that it is probable that long-chained fatty acid molecules are formed in the body by the condensation of aldehydes with pyruvic acid. We shall see later (p. 133) that both aldehyde and pyruvic acid are intermediate products in the metabolism of carbohydrates, so this gives us a clue to the method of

formation of fat from the carbohydrates of the diet—a process to which we have already referred.

The Lipoids—Lecithin and Cholesterol.

We have briefly mentioned the occurrence of lecithin and cholesterol in tissue fat, and shall now deal with them in slightly more detail before bringing the present chapter to a close. Of these substances we shall deal first with **lecithin.** This is a substance which has a widespread distribution in practically all cells and tissues, from which it can be extracted with ether or alcohol of appropriate strength. It was first obtained in this way from yolk of egg, and from this fact received its name (Gr. λεκιθος= yolk of egg). The chemical structure of lecithin is fairly well known, for the substance is readily hydrolysed on boiling with dilute alkalis. In this it resembles the true fats. And further, the products are one molecule of glycerol, one each of stearic and oleic acids, and in addition a molecule of phosphoric acid and one of a basic substance called **choline.** This last-named base is a simple substance whose constitution is well known. It is a substituted ammonium hydroxide in which three of the four hydrogen atoms are replaced by methyl groups and the remaining one by an hydroxy-ethyl group—

$$\left.\begin{array}{l} H \\ H \\ H \\ H \end{array}\right\rangle N—OH \qquad\qquad \left.\begin{array}{r} CH_3 \\ CH_3 \\ CH_3 \\ HO.C_2H_4 \end{array}\right\rangle N—OH$$

ammonium hydroxide

choline
(hydroxy-ethyl tri-methyl
ammonium hydroxide)

It is supposed that in the molecule of lecithin the stearic acid and the oleic acid are combined with two of the alcohol groups of the glycerol molecule, while the phosphoric acid is combined with the third. But phosphoric acid being a polybasic acid, has also a valency, by means of which it can unite with the choline thus:—

```
         / stearic acid
glycerol—oleic acid
         \ phosphoric acid
                    \ choline
```

The fundamental resemblance between this structure and that of a simple fat is at once seen.

This lecithin is but one member of a group of similar substances which differ at least in the fatty acids and base which they contain. As they all contain phosphorus they are referred to as the **Phospholipines** or **Phosphatides.** Less well-defined phospholipines have been isolated from the brain; they occur also in other organs, such as heart, liver and kidney.

The great importance of these phospholipines, apart from their association with the tissue fat, is that they appear to make up the framework which supports the protein constituents of cell protoplasm. They are not soluble in water in the strict sense, but yet they are capable of taking up a considerable proportion of it, and in this way they seem to confer on protoplasm the typical semi-fluid but fairly rigid consistence which is so characteristic of living matter, and furthermore, to determine the permeability of the protoplasm for dissolved salts and drugs.

Cholesterol is the chief member of a group of substances similar to itself which have been isolated from various plant and animal tissues, and are collectively known as

the **Sterols.** It was first isolated from gall stones, and received its name from the fact of its occurrence in bile (Gr. $\chi o \lambda \eta$ = bile). But it is of very widespread occurrence in the body generally, and is contained in considerable amount in the white medulla of nerve fibres. Being soluble in many organic solvents cholesterol is therefore readily prepared by extracting brains with such a solvent as acetone. It crystallises in flat rhombic plates which usually have one corner broken out in a rather characteristic fashion. Cholesterol is readily converted into a number of highly coloured products, the formation of which serves to identify the substance. Of such reactions we might mention the violet colouration, rapidly changing to a bluish green, which is observed when a solution of cholesterol in chloroform is treated with acetic anhydride and a little strong sulphuric acid. It is characteristic also that crystals of cholesterol irrigated on a microscope slide with strong sulphuric acid turn red round their edges; the addition of a little iodine then causes the crystals to turn dark violet, and eventually black. The formula for cholesterol is not settled; it is probably either $C_{27}H_{43}.OH$ or $C_{27}H_{45}OH$. It is certainly a monovalent alcohol, and as such it forms esters, which are found, for example, in the lanoline obtained from sheep's wool. But the nature of the nucleus of the molecule has not been made clear, for cholesterol, unlike fats and lecithin, is not hydrolysed into simpler products when heated with alkalis.

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Structure and Significance of the Phosphatides. (*Physiological Reviews*, Vol. I. (1921), p. 327.)

The oxidation of fats is dealt with by Dakin in his monograph on *Oxidations and Reductions*, to which reference has previously been made.

CHAPTER IX.

THE CHEMISTRY OF THE CARBOHYDRATES: THE DIGESTION OF STARCH AND SUGAR.

> "The chemistry of the carbohydrates has become a problem in geometrical permutations, and almost all the possible permutation have been identified, and most of these synthesised."—*Leathes.*

ROUGHLY speaking, we may say that the group of carbohydrates includes the sugars and starches. It is hardly necessary to point out that the use of the name carbohydrate does not imply that these substances are compounds of carbon and water; the term indicates only that the molecules of the starches and sugars contain two hydrogen atoms to every oxygen atom, so that we can say that a carbohydrate molecule has the same total composition as a mixture of carbon atoms and water molecules. To take a definite example—one of the most familiar sugars with which the student is aquainted is glucose, whose formula is $C_6H_{12}O_6$. Now this molecule can be represented as $6C+6H_2O$, but this merely refers to its ultimate composition, and not to its chemical structure, for the hydrogen and oxygen atoms in the glucose molecule are not combined as water.

Of the carbohydrates which are of interest from our point of view we will deal first with the sugars. These fall into two main groups. There are those sugars whose molecules, while differing in the actual arrangement of their constituent atoms, can all be represented as $C_6H_{12}O_6$. These make up the group of the **monosaccharides. Glucose** and **Fructose*** are the most important examples of this group.

* Glucose is sometimes known as **dextrose**, or yet again as **grape sugar.** It is important for the student to realise that these three names refer to one and the same substance. In a similar way **laevulose** and **fruit sugar** are alternative names for fructose.

Then, secondly, we have sugars whose molecules contain twelve carbon atoms, their formula being $C_{12}H_{22}O_{11}$. These are termed **disaccharides.** To this group belong **cane sugar, maltose** (malt sugar) and **lactose** (milk sugar). Each of these disaccharides is really a compound of two monosaccharide molecules united by elimination of a molecule of water, and by boiling with a little dilute acid each can be easily hydrolysed into its constituent monosaccharides. It is found in this way that

cane sugar $\longrightarrow$ glucose + fructose,
maltose $\longrightarrow$ glucose + glucose, and
lactose $\longrightarrow$ glucose + a monosaccharide called galactose.

Of course, the general equation representing this hydrolysis is $C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$. It will be noticed that cane sugar, maltose and lactose are all compounds of glucose. To complete the survey of the carbohydrates we must mention that the molecules of the starches are similarly composed of monosaccharide groups, but in relatively large numbers. For this reason they are grouped under the name **polysaccharides.** The linking up of the monosaccharide molecules to form a starch involves the loss of two molecules of water from each monosaccharide. The general formula of the polysaccharides is therefore $(C_6H_{10}O_5)n$ where n is a large number.

Before considering the behaviour of the carbohydrates in the body we must study some of their chemical properties in a little more detail. Taking the sugars as examples, the questions at once arise, How can we decide whether or not we have a sugar in solution? and, How can we distinguish between one sugar and another?

The reagent most commonly employed in testing for sugars is Fehling's solution. This is an alkaline copper solution, which when boiled with many sugars yields a red precipitate of cuprous oxide. The Fehling's solution is, as we say, *reduced* by the sugar. Incidentally, as a matter of technique, we ought to mention that Fehling's solution on standing is liable to undergo a decomposition whereby reducing substances are liberated from its own constituents. Such stale Fehling's solution will yield cuprous oxide on boiling, even in the absence of a sugar, so that it is always necessary to test the reagent first by boiling a little of it alone in order to make sure that it is fresh, before adding the solution to be tested. Now it is not all sugars which will reduce Fehling's solution. Glucose, fructose, maltose, and lactose will do so, while cane sugar will not. But of these reducing sugars, the disaccharides maltose and lactose are less powerful reducing substances than the monosaccharides, glucose and fructose. It also happens that an acid solution of copper salts is more difficult to reduce than an alkaline copper solution. Thus it comes about that while both monosaccharides and disaccharides can reduce Fehling's solution it is only the monosaccharides which can reduce a solution of copper acetate in acetic acid, which is called Barfoed's reagent. We can thus use the acid copper solution to distinguish between mono- and di-saccharides. There is little need to suggest to the student the way in which these facts can be applied to the analysis of sugar solutions. We will only mention, by way of example, that from a solution containing both cane sugar and a sugar which will reduce Fehling's solution, the reducing sugar may be removed by careful oxidation with Fehling's solution until a slight permanent blue colour indicates

excess. If the cuprous oxide be filtered off, the cane sugar remains in the filtrate. As we have said, it is incapable of reducing copper solutions, but when boiled with a little acid it is hydrolysed into a mixture of glucose and fructose, both of which are reducing sugars. Or the cane sugar may be tested for in the filtrate by utilising the fact that when boiled with strong hydrochloric acid and α-naphthol it gives an immediate purple colouration. While we are dealing with the reduction of copper solutions by sugars we ought to mention that usually these reactions are used not only as tests for sugars, but also as the basis of most of the more usual methods of estimating sugars. The percentage of a sugar in a body fluid is determined by measuring the amount of a standard copper solution which the sugar present can reduce after proteins and other interfering substances have been removed. We shall not, however, deal with the detailed technique of these quantitative methods as they are fully described elsewhere.

But we must refer to another method whereby individual sugars are identified. When a chemist has discovered the group to which a substance belongs he usually decides which particular member of that group he is dealing with by determining its melting or boiling point. In the case where a substance will not even melt without decomposition it is usual to convert it into some crystalline derivative whose melting point is sharp and definite. Now the sugars are such substances which decompose on heating, but they form yellow crystalline compounds called **osazones** with the organic base **phenyl-hydrazine,** and these osazones have definite crystalline forms and melting points by means of which they, and so their parent sugars, can be identified. Hydrazine itself is a

compound of two amino groups:—NH_2—NH_2: its phenyl derivative is $C_6H_5.NH.NH_2$. In practice, in order to make the osazone of a sugar, one takes, say, half a test tube full of the sugar solution, adds as much phenyl-hydrazine hydrochloride as will lie on a sixpenny piece and as much sodium acetate as will lie on a shilling. This last-mentioned salt acts as a "buffer," and prevents the hydrochloric acid liberated, as the phenyl-hydrazine is used up, from producing too great an increase of acidity, for this would hinder the reaction. The mixture is warmed until everything is in solution, filtered if not clear, and then heated in a boiling water-bath for half an hour. Then, on allowing the test-tube to cool slowly, the osazone of the sugar usually crystallises out. It may be purified by recrystallisation from dilute spirit.

Of the sugars we have mentioned, all except cane sugar form osazones, but the osazone derived from fructose is the same substance as that derived from glucose (phenyl-glucosazone). The reason for this is that the molecule of fructose differs from that of glucose only in the two end groups:—

$$\begin{array}{lcl}
CH_2OH & \qquad\qquad & CH_2OH \\
| & & | \\
CHOH & & CHOH \\
| & & | \\
CHOH & & CHOH \\
| & & | \\
CHOH & & CHOH \\
| & & | \\
CHOH & & CO \\
| & & | \\
CHO & & CH_2OH \\
\text{glucose} & & \text{fructose}
\end{array}$$

and as it happens, it is these two groups which are attacked by the phenyl-hydrazine in each case in such a way that

the formation of the osazone obliterates the difference of structure.

By this time the student must have been struck by the fact that many of the sugars possess the same formula; they are isomeric. Indeed, in many sugars, the very groups which make up the molecule are identical, the differences in chemical properties being due entirely to slight differences in space arrangement of the atoms. Take, for example, the monosaccharides with the formula $C_6H_{12}O_6$—some of these sugars of course differ in the groups which are present in the molecule in the way that glucose differs from fructose, but, on the other hand, there are quite a number of sugars in which the groups are exactly the same as those in the glucose molecule. In all of these there are differences in the spatial arrangement of the various groups, and the most fundamental property by means of which all these isomers may be distinguished is that which depends upon the space arrangement of atoms and is known as **optical activity.** In order to understand this property one must be, to some extent, familiar with the nature of light. It is well known that light is a form of energy which is composed of transverse waves in the all-pervading ether. This means that each ether particle oscillates in a plane which is at right angles to the direction in which the light is travelling. In this respect the light waves resemble waves on the surface of water, where it is a matter of observation that a floating particle moves up and down at right angles to the general surface, while the waves move forward along the surface. (On the other hand, in sound waves we have a case where the movement of the vibrating particles is to and fro in the same direction as that in which the sound is progressing.) Now it is evident that there are an infinite

number of planes at right angles to the direction in which a ray of light may be travelling. For example, in fig. 8 let the thick arrow represent the direction of a ray of light travelling downwards away from the reader; then one possible plane at right angles to this direction is that shown and a given ether particle might be vibrating up and down in the dotted line in the plane. On the other hand, an alternative arrangement is that shown in fig. 9 where the ether particle vibrates from side to side:

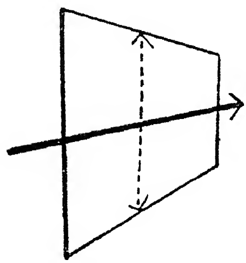

FIG. 8.

And between these two extremes any number of possible intermediate conditions exist. Now, ordinary light—even that of one colour—consists of a mixture of rays in which the vibrations of the ether particles are in all these different planes at right angles to the direction of the rays. It is possible, however,—most easily by passing the light through certain crystals, of which Iceland spar is the best known,—to filter off all the rays except those in which the vibration is in *one* particular plane. These remaining rays then form a beam of what is called polarised light. No difference is noticed between this light and ordinary daylight when they fall on the eye, but it differs with regard to its behaviour when passed through a crystalline structure, for example, a second crystal of Iceland spar. For, as we have said, it is a property of such crystals as Iceland spar to let through only such rays

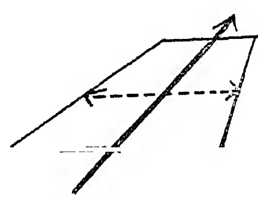

FIG. 9.

as are vibrating in one particular plane which has a definite relation to the axes of the crystal, and is known as its optical plane. If then we have a ray of this polarised light, it will pass through the crystal, when the optical plane of the crystal corresponds with the actual plane in which the vibrations are occurring, but it will not get through the crystal if its optical plane is turned at right angles to the plane of vibration of the light. In intermediate relative positions of the two planes more or less light gets through according as they are nearly coincident or more nearly at right angles. Of ordinary daylight, of course, some will be transmitted in any position of the crystal, because there will always be some rays which are vibrating in the optical plane. Now there are many substances which either in the solid or dissolved condition have the power of rotating the plane of polarisation of light, they are said on that account to be optically active. In other words, if a ray of polarised light with a certain plane of vibration passes into a solution of such a substance, then the ray when it emerges is found to have a different plane of vibration from that which it originally possessed before entering the solution. The angle through which the plane of vibration is turned depends on the particular substance used, on the strength of the solution, and the length of solution through which the ray has passed. Now suppose that we have a source of light polarised in one particular plane, and view it through a crystal of spar so arranged that its optical plane corresponds to the plane of vibration, the light will pass through in maximum amount. Now let us interpose between the source of light and the crystal a layer of some optically active substance, the plane of vibration of the light will be rotated so that when the ray emerges its

plane will no longer coincide with the optical plane of the crystal. The intensity of light seen through the crystal will, therefore, be diminished. The maximum intensity can, however, be restored by turning the crystal until its optical plane corresponds with the new plane of vibration. It will be seen that the angle through which the crystal will need to be turned will be exactly equal to that through which the optically active substance has turned the plane of polarisation. In this way we can measure degrees of optical activity. Now, it is found that optically active substances contain in their molecules, as a rule, at least one carbon atom which is united to four different groups. Such an **asymmetric carbon atom** occurs, for example, in lactic acid, and this is an optically active substance. This can be seen if the formula is written as follows:—

$$\begin{array}{ccccc} & & \mathrm{OH} & & \\ & & | & & \\ \mathrm{CH_3} & — & \mathrm{C} & — & \mathrm{COOH} \\ & & | & & \\ & & \mathrm{H} & & \end{array}$$

where the four different groups attached to the central carbon atom are $-CH_3$, $-H$, $-OH$ and $-COOH$. The sugars usually contain several asymmetric carbon atoms in each molecule. In glucose, for example, by writing the formula at length:—

$$\begin{array}{ccc} & \mathrm{CH_2OH} & \\ & | & \\ \mathrm{H}— & {}^*\mathrm{C} & —\mathrm{OH} \\ & | & \\ & \mathrm{(CHOH)_3} & \\ & | & \\ & \mathrm{CHO} & \end{array} \quad \begin{array}{ccc} & \mathrm{CH_2OH} & \\ & | & \\ & \mathrm{CHOH} & \\ & | & \\ \mathrm{H}— & {}^*\mathrm{C} & —\mathrm{OH} \\ & | & \\ & \mathrm{(CHOH)_2} & \\ & | & \\ & \mathrm{CHO} & \end{array} \quad \begin{array}{ccc} & \mathrm{CH_2OH} & \\ & | & \\ & \mathrm{(CHOH)_2} & \\ & | & \\ \mathrm{H}— & {}^*\mathrm{C} & —\mathrm{OH} \\ & | & \\ & \mathrm{CHOH} & \\ & | & \\ & \mathrm{CHO} & \end{array} \quad \begin{array}{ccc} & \mathrm{CH_2OH} & \\ & | & \\ & \mathrm{(CHOH)_3} & \\ & | & \\ \mathrm{H}— & {}^*\mathrm{C} & —\mathrm{OH} \\ & | & \\ & \mathrm{CHO} & \end{array}$$

we can show that four of the carbon atoms are asymmetric. We do not wish to go into details at this point and to discuss all the possible space arrangement of the groups which occur in this typical monosaccharide molecule, we wish merely to point out that it is the possession of these asymmetric carbon atoms that confers on the sugars this property of optical activity. The reasons why we have dealt with the subject at such length are, firstly, that the actual value of the optical activity of a sugar is a very valuable constant whereby it can be identified; and, secondly, that if the particular sugar present in a solution be known, then the strength of the solution can be determined by a measurement of the optical rotation. This fact finds much practical application in breweries and sugar refineries where the estimation of the percentage of sugar in a fermenting liquid or in treacle would be more difficult to carry out by any other method.

We ought to mention that some optically active substances turn the plane of polarisation to the right—these are called dextro-rotatory substances—while others turn the plane to the left,—these being termed lævo-rotatory. Glucose and cane sugar are dextro-, and fructose is lævo-rotatory. It is found that an optically active substance always exists in two forms, one of which is dextro-rotatory, and the other is to an equal extent lævo-rotatory. Thus there are such *d*- and *l*-forms of lactic acid, of glucose, tartaric acid, and so on. The existence of these two forms is accounted for by the circumstance that if the asymmetric carbon atom of a molecule be regarded as situated at the central point of an imaginary tetrahedron, and the four different groups be supposed to occupy the four corners of the figure, i.e. to

be equally distributed round the central carbon atom in the three dimensions of space, then by interchanging any two of the groups a new molecule is obtained which is still composed of the same radicles united in the same way, but with an arrangement in space which differs from that possessed by the original molecule as the mirror image of an object differs from the object itself, or the one member of a pair of gloves differs from the other. The two formulæ can never be made to coincide group for group, and they thus stand for distinct chemical substances which, while agreeing in their general reactions, differ in one or two respects, the chief of which is the direction of rotation of the plane of polarisation of light. For this reason such a pair of substances are referred to as optical isomers. A substance prepared in the ordinary way in the laboratory is usually obtained as a mixture of the two isomers which is optically inactive. By various methods this mixture may be resolved into its two optically active components. On the other hand, the same substance, if it occurs in nature as a product of plant or animal metabolism, is usually in the form of only one isomer. Thus lactic acid synthesised in the laboratory is always found to be inactive, while that obtained from muscle is composed entirely of the dextro-rotatory isomer.

We have yet to deal with the polysaccharides. Of these, ordinary vegetable starch has been cited as the most familiar example. It is an amorphous substance which is soluble in boiling water, forming a clear solution, The most delicate test for it is the intense dark blue colour which it gives with iodine. Starch can be precipitated completely from solution by half-saturation with ammonium sulphate.

The digestion of starch is an easy process to follow.

This substance is attacked first of all by the **ptyalin** of the saliva (Gr. πτυω, I spit). This amylolytic enzyme breaks the starch down into dextrin molecules, which are polysaccharides of smaller molecular weight. These dextrins are broken down still further into simpler dextrins, and these finally are resolved into maltose molecules. This process of hydrolysis can be easily followed in a test tube in which some saliva is added to a little starch paste. If the mixture be kept warm the digestion proceeds rapidly, and as it progresses it will be found that as the starch becomes used up small drops of the mixture removed at intervals from the main bulk and tested with iodine give less and less of the typical blue colour; when the more complex dextrins are present it will be found that a red colouration is produced, and at the end, when only the simpler dextrins and maltose remain, the tested drop will give no colour with iodine at all. In the mouths of even those of us who are most fastidious with regard to chewing, very little more occurs than the mixing of the starch and the saliva. The main portion of the salivary digestion occurs while the food is lying in the stomach. Not that ptyalin can act in an acid medium,—indeed it is destroyed by a degree of acidity as great as that which obtains in the stomach—but the diffusion of the acid into the food is sufficiently slow for the ptyalin to remain unharmed in the middle of the mass for as much as half an hour after it is swallowed. The next enzyme to attack the starch is the **amylase** of the pancreatic juice; this continues in the duodenum the work already begun by ptyalin. As a result we have in the intestinal contents all the maltose obtained by the breaking down of the starch of the food, together with any other disaccharides such as cane sugar or milk sugar which may have been taken, and which so far

have remained unchanged. In the intestinal juice we find a series of three enzymes which bring about the hydrolysis of these disaccharides to their respective monosaccharides, as has been already described (page 111); there is a **maltase** which brings out the conversion of maltose into glucose, a **lactase** which liberates glucose and galactose from lactose, and, lastly, an **invertase** which inverts the cane sugar, liberating glucose and fructose. This last enzyme derives its name from the fact that while cane sugar is dextro-rotatory the mixture of glucose and fructose derived by its hydrolysis is lævo-rotatory, the lævulose producing a more powerful left-handed rotation than that produced in the opposite direction by an equal concentration of glucose. The sign of the rotation of a cane sugar solution is therefore inverted on hydrolysis.

It is in the form of these monosaccharides that the fully digested carbohydrates are absorbed into the blood stream. In this study of these processes of the digestion of carbohydrates we have another illustration of the principle that it is necessary not only to bring the food into a soluble and diffusible form in which it can be absorbed from the alimentary canal and carried to the tissues, but that it is necessary also to bring it into some form which also can be utilised by them. Take the case of cane sugar. It is useless to the tissues. They contain no enzyme which can break it up, and if cane sugar is injected into the blood stream it travels round the circulation as a foreign body until it becomes excreted by the kidney, and so wasted. Glucose is no more soluble or diffusible than cane sugar, but it is **assimilable,** so that any glucose which enters the blood is, in general, oxidised in the body and does not appear in the urine. It is just

as important, therefore, to break down a molecule of a disaccharide into assimilable monosaccharide molecules as to break down the most complex of proteins into its constituent amino-acids.

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Directions for practical work on these substances are given in the volumes by Cole and Plimmer, to which we have already referred in previous chapters.

CHAPTER X.

THE UTILISATION OF CARBOHYDRATES: THE CHEMICAL MECHANISM OF MUSCULAR CONTRACTION.

"The lactic acid is part of the machine and not part of the fuel."
—*A. V. Hill.*

We have just seen that the carbohydrates of the food are broken down to their constituent monosaccharides, and are absorbed in this form into the blood stream. Now we must study the uses of these substances in the body. It may be stated at the outset that the chief and most characteristic of these uses is as fuel. This follows at once from the simple observation that during the activity of a muscle its stores of carbohydrates diminish. So that one of the chief subjects with which we shall have to deal in connection with the metabolism of carbohydrates will be the way in which these substances are oxidised—ultimately to carbon dioxide and water—with the liberation of energy, partly in the form of heat and partly as the mechanical energy of movement.

But before we proceed to this main discussion we will point out that in the mammalian body there is abundant provision for the storage of excess carbohydrate material derived from the food, until it is required. In this there is a resemblance to the behaviour of the body towards fat which, as we have seen, is so readily stored in the tissues, and a marked contrast to its behaviour towards

the nitrogen of proteins, excess of which cannot be stored under ordinary conditions. On absorption from the intestine the monosaccharides formed by the disruption of more complex carbohydrate molecules are carried in the portal blood stream to the liver. Here a very considerable amount of storage takes place, the monosaccharide molecules being condensed, under the influence of an enzyme called glycogenase, to form the polysaccharide **glycogen,** or animal starch, which is deposited in the liver cells in granules. This glycogen is a white amorphous substance which dissolves in hot water, forming an opalescent solution. It differs from vegetable starch, in giving a red colour with iodine, but, like vegetable starch, glycogen breaks up into glucose molecules on hydrolysis. This hydrolysis occurs in the body as soon as the percentage of sugar in the blood falls below normal. The store of glycogen in the liver is then gradually drawn upon to maintain a normal amount of sugar in the blood, as, for example, during a period when glucose is not being absorbed from the alimentary canal. It should be mentioned that the liver is not the only organ where a store of glycogen is laid down. The muscles themselves contain a considerable amount of reserve carbohydrate in this form. Whenever these reserves are to be drawn on, the glycogen is first hydrolysed to glucose (under the influence of the same enzyme, glycogenase, which controlled its formation) and then transported in the dissolved condition to places where the fuel is required.

We have already mentioned that carbohydrates may also give rise to fat, and may be stored in this form (p. 101).

Returning now to our main theme—the utilisation of carbohydrates for the obtaining of energy in the muscles. The one fundamental fact which must at first be stated

is that the fuel is not consumed by a muscle during the moment of its contraction. The contraction of a muscle is a process which itself is not accompanied by the absorption of oxygen or the evolution of carbon dioxide. The energy for the contraction is obtained by the sudden breaking down of some unstable complex molecule in the muscle substance into simpler fragments, when a nervous impulse arrives; but this disintegration does not involve an oxidation. It used to be thought that the energy liberated during a muscular contraction was obtained from a kind of explosive combustion which is fired off by the nervous impulse. But seeing that a muscle will contract in an atmosphere free from oxygen—say, in nitrogen gas—it is evident that any oxygen required for such an explosive reaction must be derived from some source in the muscle itself. It was therefore supposed that oxygen was actually stored in the muscle as one part of a complex molecule, of which other portions were composed of combustible material. It was imagined that when the muscle was stimulated this combustible material was burnt up in the "intra-molecular oxygen," and that the energy for the contraction was thereby obtained. But we know now that when a muscle contracts in nitrogen, no carbon dioxide is produced,—or rather, that any carbon dioxide which escapes from the muscle has been liberated from the carbonates which the muscle originally contained before it contracted. **The act of contraction, therefore, is not necessarily accompanied by the evolution of carbon dioxide; it therefore does not involve an oxidation of fuel.** But as a result of contraction **lactic acid** appears in the muscle, and the more powerful the contraction the more lactic acid is produced. When a muscle goes into heat rigor it contracts to its utmost

extent, and at the same time it liberates its maximum amount of lactic acid. Now this lactic acid which is suddenly set free in the muscle in this way is of extreme importance, for it is supposed to cause the actual shortening of the contractile substance by one of several possible methods which we shall mention later. This lactic acid is evidently formed *without oxidation* from some substance in the muscle at the moment when the muscle sets free a large amount of energy. Probably the lactic acid comes, immediately, from glucose, for one glucose molecule contains just those atoms which are required for producing two molecules of lactic acid:—

$$\underset{\text{glucose}}{C_6H_{12}O_6} \longrightarrow \underset{\text{lactic acid}}{2C_3H_6O_3}.$$

But this change in itself is not accompanied by the liberation of any great amount of energy, so that if the lactic acid is actually produced from glucose in the first instance, this glucose must itself form part of an unstable complex which liberates a large amount of energy when it breaks down. It has been shown that during this change, the conversion of the potential energy of this original complex into the energy of tension in the muscle is very efficient, for very little energy is wasted as heat. But whether the tension developed in the muscle is converted into useful work or not depends entirely on the way in which the muscle is loaded; if it is not allowed to shorten at all, then all its tension energy will be converted into heat; if it can pull up a weight, then useful mechanical work will result. But even so, no matter how appropriate the amount of load, always a certain amount of the energy will be converted into heat and lost. But this is a topic which cannot be discussed in detail here.

The lactic acid which has been liberated, and which has produced the shortening of the contractile substance, remains in the muscle if this is in an atmosphere devoid of oxygen; if further contractions are brought about, then the extra quantities of lactic acid produced by these are also added to the first, so that the acid accumulates. This accumulation of lactic acid in the muscle is associated with the appearance of fatigue. Fatigue is a complex phenomenon even in a single isolated muscle-nerve preparation. The relation of the acid to the onset of fatigue may be briefly stated to be threefold; the acid diminishes the ease with which the excitation passes from muscle to nerve to bring about the initial stages in the contraction; then it diminishes the power of the contractile substance to respond to further quantities of lactic acid which may be liberated; and, lastly, the lactic acid which has been produced represents a breakdown product of the original complex molecule from which the energy of the contraction is derived. The fatigued muscle will, therefore, contain less of the energy-laden complex, and will, as a result, have an ever-diminishing store of potential energy for subsequent contractions.

But it is not with fatigue that we are so much concerned at the moment, as with the ultimate fate of the lactic acid. It is found that if oxygen be admitted to the muscle after the contraction, its lactic acid disappears, and carbon dioxide is produced. There has been a considerable discussion as to the way in which this removal of the lactic acid is brought about. It has been suggested that the acid is simply oxidised to carbon dioxide and water, but the evidence is rather in favour of the view that most of the acid is built back into the "glucose-complex," from which it split off when the muscle was stimulated. For it is

found that during the processes of restitution in an oxygenated muscle after its contraction, as the lactic acid disappears the total amount of carbohydrate to be obtained from the muscle increases. In other words, the lactic acid represents an essential part of the chemical machine, and is used over and over again to produce contractions. Of course, in order to replace the lactic acid into the original energy-containing complex new energy must be supplied—and this energy comes from the oxidation of fuel in the muscle. That the fuel used by the muscle during restitution is carbohydrate is shewn by the fact that during this process the volume of oxygen absorbed by the muscle is equal to the volume of carbon dioxide produced by it, as must necessarily be the case when carbohydrate is being oxidised (see p. 157). The energy of combustion of this carbohydrate is not all converted into potential energy in the muscle; a certain amount of it appears as heat, but less heat is given off during the restitution than the total amount which could be obtained from the oxidation of the quantity of carbohydrate which is observed to disappear. The deficit represents the amount of energy required for restoring the muscle to its former energy-laden condition. From this account it will be realised that the main energy-yielding oxidations in a muscle occur *after* any one contraction and in preparation for the next. The muscle is a kind of machine which, in its resting state, is wound up, so to speak, ready to liberate energy, without the occurrence of oxidations, as soon as its mechanism is released by a nervous impulse. It is for the rewinding of the machine, for the replacement of its previously liberated energy, that the oxidation of fuel is required. The muscle differs, therefore, very considerably from a steam engine. For

in the steam engine the oxidation of the fuel and the liberation of the energy go hand in hand. The steam engine must have oxygen *while* it is working. On the other hand the muscle requires oxygen *after* it has worked, in order to fit it for its subsequent activity. It is as if the compressed steam in a boiler could be stored indefinitely, be drawn up as required to drive the mechanism of the engine, and be replaced subsequently during a period of rest of the engine by the combustion of fuel. We should then have a condition in which the oxidation of fuel would occur not during the utilisation of the energy, but after—a condition which is the reverse of that which usually obtains in a steam engine, but which, as we have seen, is found in a muscle.

We have said that the fuel used by the muscle is usually carbohydrate, but we should mention also that cases are known in which a muscle has been shown to be using fat as a fuel in its restitution stage.

In this description of the chemical mechanism of muscular contraction we have supposed that the muscle under consideration has been made to contract in an atmosphere of nitrogen, and that afterwards oxygen has been admitted to it in order to permit of its restitution. But the student will have realised that, in the case of a muscle receiving during its activity in the body an efficient oxygen supply, the processes of contraction and restitution go forward simultaneously. We have adopted our present mode of treatment of the subject merely in order to show that contraction *can* be brought about in the absence of oxygen, and that, therefore, it is a process which does not involve an oxidation, and is thus distinct from the process of restitution which does.

There now remain two further very important problems

with regard to the muscular mechanism with which we must briefly deal. In the first place we must consider how the lactic acid which is suddenly liberated when a muscle is stimulated actually produces the tension or the shortening which is observed. Several ways in which this effect might possibly be produced have been suggested. For example, it has been supposed that the acid produces a swelling of the colloidal constituents of the muscle fibres, so that each fibre increases in diameter, but shortens at the same time. Or it may be that the acid produces some change of osmotic pressure of the muscle contents, or of permeability of some membrane in the fibre in such a way that water is drawn in osmotically, and produces the swelling and the tension. The way in which such a process would work will be clear if the student will refer to the account of osmotic pressure in the last chapter. Lastly, there is the further possibility that the immediate source of the mechanical energy is a change of surface tension at some surfaces in the muscle fibres. In dealing with the functions of bile in the digestion of fats we have already described how, owing to intermolecular forces the area of a liquid surface or interface tends to become as small as possible. We may suppose that the acid suddenly liberated in the muscle fibre produces a correspondingly sudden increase in the tension at some surface or surfaces in the fibre, and that it is the sum of such separate increments of tension that constitutes the total pull of the whole muscle. This view of muscular activity is supported by the observation that the total amount of energy liberated during the contraction of a muscle is proportional to the initial length of its fibres in the resting condition. On our present theory this is easy to explain, for the increase in length

means increase in area of the surfaces, so that if the same increase of energy per unit area of surface occurs on stimulation, the total energy liberated will be the greater, the greater the surface over which the increase is operative. This effect is observed not only in the case of voluntary muscle, but also in the case of cardiac muscle; in fact, it constitutes one of the most important means whereby the heart is enabled to increase the energy of its contraction when there is a demand for increased efficiency of the circulation.

The second and last of our important problems relating to muscle is concerned with the details of the steps in the oxidation to carbon dioxide and water of the glucose which supplies the energy for the restitution of the muscle. This is a subject on which we have not certain and complete information at the present time. The main reason for this lies in the difficulty of identifying directly the intermediate products of this, as of many bio-chemical reactions, because they have so temporary and fleeting an existence, and are converted into further products before they have time to accumulate in any quantity. There is no doubt, however, that during the oxidation of the glucose molecule for the obtaining of energy with which the restitution of a muscle is brought about, lactic acid is an intermediate product. This lactic acid of course has a different origin from that which forms part of the actual muscular mechanism and which is liberated by the disintegration of the "glucose complex." The formation of lactic acid from glucose which is being used as a fuel is shown by its accumulation in the circulating blood when muscular work of such intensity is being performed that the oxidation processes of the body cannot burn the

fuel completely to carbon dioxide and water. Now it is found that most tissues contain a ferment **glyoxalase** which has the power of producing lactic acid from **methyl-glyoxal,** thus:—

$$
\begin{array}{ccc}
CH_3 & & CH_3 \\
| & & | \\
CO & \longrightarrow & CHOH \\
| & & | \\
CHO & & COOH \\
\text{methyl-glyoxal} & & \text{lactic acid}
\end{array}
$$

We thus arrive at the conclusion that probably methyl-glyoxal and lactic acid are successive steps in the break-down of the glucose molecule. The later stages are, perhaps, more difficult to make out. It is supposed, however, that pyruvic acid is the next product in the series:—

$$
\begin{array}{ccc}
CH_3 & & CH_3 \\
| & & | \\
CHOH & \longrightarrow & CO \\
| & & | \\
COOH & & COOH \\
\text{lactic acid} & & \text{pyruvic acid}
\end{array}
$$

It will be remembered that we have already met pyruvic acid as the product of the deamination of alanine (p. 48); it is an important substance on account of its occurrence as a stage in the utilisation of amino-acids as well as of carbohydrates. Furthermore, it is probably the starting point from which the body synthesises fat from carbohydrates. The next probable step in the oxidation involves a reduction of the carbon chain to two atoms, by the loss

of a molecule of carbon dioxide from the carboxyl group, acetaldehyde being thereby produced:—

$$\begin{array}{ccc} CH_3 & & CH_3 \\ | & & | \\ CO & \longrightarrow & CHO \\ | & & \text{acetaldehyde} \\ COOH & & \\ \text{pyruvic acid} & & \end{array}$$

This acetaldehyde is then further oxidised,—probably first to acetic acid, and then finally to carbon dioxide and water.

One is reminded, in this discussion of the metabolism of glucose in the animal body, of the fermentation of glucose to alcohol and carbon dioxide by yeast. But there are fundamental differences between the course followed in the breakdown of glucose in a yeast and in a muscle cell respectively. For one thing, the initial stages of alcoholic fermentation do not involve the formation of lactic acid, for the yeast cell cannot ferment lactic acid as it should be able to do if this substance were a normal intermediate stage in the production of alcohol from sugar. Then, secondly, although alcohol can be oxidised in a muscle, it does not seem likely that it occurs as a normal breakdown product of glucose in the body; whereas it is the end product of the fermentation produced by yeast.

SUMMARY.

It may assist the student to realise the chemical mechanism of muscular contraction, if we present him with the following comparison between the events occurring during a muscle twitch and those which take place during a single stroke of the engine of his motor bicycle. The chief point to be emphasised is the difference in the relative time at which the combustion processes take place in the muscle and in the engine respectively.

MUSCLE.	PETROL ENGINE.
Nervous impulse arrives.	Ignition spark passes.
Lactic acid is liberated from its precursor. During this process no combustion takes place, but a small quantity of heat is liberated.	**Combustion of the fuel occurs.** Heat is liberated and carbon dioxide and water are produced.
The lactic acid acts on the contractile structure of the muscle fibres, with the result that tension is set up.	The heated gases in the cylinder set up a compression.
The energy of the tension is converted partly into mechanical work and partly into heat in relative amounts determined by the conditions of loading.	The energy of compression is converted partly into mechanical work and partly into heat in relative amounts according to the load on the engine.
The muscle fibres relax. This process involves the further production of heat from the internal friction of the muscular structure.	The piston returns to its initial position in the cylinder, a certain amount of heat being produced by friction.
Combustion of carbohydrate fuel now takes place. Of the energy so produced a part is utilised for building back most of the lactic acid into its precursor; the remaining energy is liberated as heat.	Fresh supplies of fuel and air are drawn into the cylinder.

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On the stages in the oxidation of glucose:

Dakin's Monograph on *Oxidations and Reductions*,

and the more recent article by the same author entitled:

Physiological Oxidations. (*Physiological Reviews*, Vol. I. (1921), p. 394.)

Those who wish to study the intermediate stages in the production of alcohol from sugar should consult the volume:

Alcoholic Fermentation, by A. Harden.

This is one of the Monographs on Biochemistry.

CHAPTER XI.

THE PATHOLOGY OF CARBOHYDRATE METABOLISM: GLYCOSURIA: DIABETES.

"If the carbohydrate fires do not burn briskly enough, the fat is incompletely consumed; it smokes, as it were, and the smoke is represented in metabolism by the ketones and derived acids."—*Macleod.*

ONE of the most important aspects of the metabolism of carbohydrates is furnished by the study of the commonest pathological condition in which it is deranged, namely, **diabetes.** In this disease the fundamental observation which one makes is that the blood and also the urine contain an abnormal amount of glucose. There seems to be the minutest trace of sugar in normal urine, but in diabetes this is much exceeded, and quite appreciable quantities are to be found. The harmful results of diabetes, however, are not due to the mere wasting of this excess of sugar, but rather to the circumstance that disturbance of the carbohydrate metabolism usually involves serious derangement of the other lines of metabolism also occurring in the body. These we shall describe presently; for the moment we shall turn attention to the chief causes which lead to the appearance of sugar in the urine. We must mention that the behaviour of the kidney towards the sugar in the blood is rather a special one. The normal concentration of glucose in the blood is a few tenths per cent. If only this amount of sugar is present in the circulation, then, as we said, practically no sugar appears in the urine; but if the concentration of

sugar in the blood is increased, when a certain concentration is reached, the sugar leaks over into the urine. Thus the concentration of sugar in the blood has to reach a certain **threshold** value before it begins to pass the kidney. The behaviour of the kidney towards glucose is therefore very different from its behaviour towards such a substance as urea, which always appears in the urine, no matter how small its concentration in the blood. On account of this, urea is termed a **non-threshold body,** as opposed to the threshold bodies like glucose. There are two possible ways in which this mechanism can be upset; either the kidney may be damaged in some way so as to interfere with its power of keeping back the sugar, when this is present in normal amount, or the concentration of sugar in the blood may become so far increased above the threshold value that the normal kidney cannot retain it. With regard to the first of these disturbances—the damage to the kidney—we find that there is a drug called **phloridzin** which has the power of disturbing the normal permeability relationships of the kidney for sugar, and under its influence the kidney allows the sugar to escape even when it is below its normal threshold concentration in the blood. This furnishes one way of causing sugar to appear in the urine. On the other hand, the easiest way for satisfying the second condition and increasing the concentration of sugar in the blood above the normal threshold value is to eat a large excess of sugar. It is not of much use eating starch and other carbohydrates which give rise to sugar during digestion, because the hydrolysis and absorption of these substances is sufficiently slow to allow of the sugar being dealt with by the liver, but if considerable quantities of glucose are eaten then it swamps the liver and cannot

be dealt with sufficiently quickly to prevent an abnormal concentration of sugar in the blood, which leads to the appearance of sugar in the urine. This condition is called **alimentary glycosuria.**

There is another very important way in which glycosuria can be produced. It was discovered by Claude Bernard, and called by him **diabetic puncture.** Claude Bernard hit upon the idea of stimulating the nucleus of the vagus nerve and observing the effect on the formation of glycogen in the liver. In order to do this he stuck a pair of electrodes into the floor of the fourth ventricle of the brain, and was surprised to find that this procedure gave rise to glycosuria, even in the absence of any electrical stimulation. On working out the physiology of the effect, he found that it was due to a condition of hyperglycæmia—to such an increase of the sugar in the blood that the kidney could not keep it back. This increase of blood sugar is due to the rapid hydrolysis of the glycogen of the liver, brought about not by impulses passing along the vagi, but by impulses which pass down the spinal cord from the point of puncture of the medulla and out by the splanchnic nerves; for if the splanchnics be cut, the effect is not observed. It is found, however, that the adrenal bodies must also be intact; if they are removed, then diabetic puncture fails. This is probably because the splanchnics are secretory nerves to the adrenal bodies, causing the liberation of adrenalin into the blood. This may act directly as a stimulus to the other splanchnic endings in the liver, or, at least, may render them more sensitive to the nervous impulses passing to that organ, and so lead to a considerable turning out of the glycogen. As a matter of fact, a large injection of adrenalin is found to stimulate the splanchnic endings in the liver, and so to

produce an effect which is very similar in fundamental respects to that of diabetic puncture.

Lastly, and most important of all, we have what is called **pancreatic diabetes.** It is a condition of hyperglycæmia and consequent glycosuria which results from removal of the pancreas from an animal. It is found that if the pancreas be cut out from a dog, then in a very short time after the operation the animal developes severe diabetes. That this is not due to a loss of pancreatic juice from the duodenum is shown by the observation that mere ligature of the duct will not give rise to diabetes; nor will this condition develope if the pancreas be removed from its normal position and sewn up into the subcutaneous connective tissue, provided that to the grafted organ a good blood supply is maintained. We can be quite sure, therefore, that it is some **internal secretion** or **hormone,** produced by the pancreas, which is responsible for keeping the carbohydrate metabolism normal. This is also shown by the observation that in a de-pancreatised pregnant bitch the internal secretion of the pancreases of the embryos diffusing across the placenta into the maternal blood is sufficient to protect the mother from diabetes until the pups are born and her supply of the hormone consequently fails. The student who is familiar with the histology of the pancreas will remember that it is composed of two kinds of tissue. There are the ordinary glandular alveoli lined by the cells which produce the pancreatic juice—the external secretion—and also a certain amount of tissue consisting of masses of cells not arranged in alveoli, which cell masses have been named **islets of Langerhans.** These islets have a particularly good blood supply, and therefore are well situated with regard to the formation of an internal secretion. It has been shown,

as a matter of fact, that these islet cells are quite distinct in function from the ordinary cells which form the external secretion. The internal secretion produced by the islets of Langerhans, and passing from them into the blood, seems to be essential for the burning up of glucose in the tissues in the way we outlined in the last chapter. If that internal secretion be not present, then the tissues no longer have the power of using glucose as a fuel; the result is that it accumulates in the blood and so gets over into the urine. Meanwhile the stored glycogen of the body is drawn upon—the tissues, as it were, call for it—but this is of no avail, for the glucose coming from the glycogen can no more be utilised than that which comes from the alimentary canal. The next thing that happens is that the tissue proteins begin to break down at an abnormal rate, so that the keto-acids derived from them may be used as a source of glucose. We know this from the fact that the ratio of glucose to the total nitrogen of the urine settles down to a constant value. If a molecule of protein is giving rise to, say, D molecules of glucose (dextrose), and at the same time to N molecules of nitrogen in the form of urea, then of course the ratio $\frac{D}{N}$ will necessarily be constant, provided that a constant fraction of the glucose is excreted and that it arises from no variable source except protein. The glucose and nitrogen arise from the same initial substances, and therefore their amounts must necessarily be proportional to each other. The result of this excessive production and non-utilisation of glucose means that the blood becomes swamped with it until it cannot be kept back by the kidney, and so appears in the urine.

Human diabetes possesses most of the characteristics of this pancreatic diabetes, although its presence is not

invariably associated with visible pathological changes in the pancreas itself. As we have pointed out, the chief danger of the disease is not merely that sugar is wasted from the body, but rather that the sugar has been derived from the proteins of the tissues. It is the loss of the nitrogen, rather than that of the sugar, which is of greatest consequence. Then, again, other serious symptoms of the disease result from the circumstance that when sugar is not being oxidised, the oxidation of fats is also interfered with and becomes incomplete. Instead of being burnt right up to carbon dioxide and water, the fatty acids remain in a half-oxidised condition in the form of **aceto-acetic acid,** $CH_3CO.CH_2.COOH$, and **β-oxy-butyric acid** $CH_3CHOH.CH_2COOH$. These substances are poisonous, firstly, because they are strong acids, and so disturb the neutrality regulation in the tissues. Evidence of this disturbance is shown by the increased ammonia contained in the urine. Whenever abnormal amounts of acid appear in the blood, some of the ammonia, which otherwise would be converted to urea in the liver, is kept over to neutralise the harmful acid. Further, the tension of carbon dioxide in the alveolar air is reduced, because the hydrogen-ion concentration of the blood becomes increased, so that there is more powerful stimulation of the respiratory centre, and therefore, increased ventilation of the lungs, with a consequent more complete washing out of the carbon dioxide. This reduction of the carbon dioxide content of the alveolar air—and so of the carbonic acid content of the arterial blood—constitutes a further means whereby the increased acidity due to the foreign acids may be partially compensated. But not only are these substances poisonous because they are acids, for even in the

form of sodium salts they seem to have a distinct poisonous action. As the disease progresses they accumulate in the blood, and eventually poison the nervous system, bringing on a condition of coma, which is almost invariably fatal. We have said that these acids are produced from fats whenever carbohydrate metabolism is interfered with; it is not surprising, therefore, to find them produced, not only when the tissues are rendered incapable of oxidising glucose, but also when the tissues are robbed of carbohydrates by withholding these substances from the food. Under these circumstances we have no carbohydrate oxidised, simply because there is none to oxidise, and the result is the production of aceto-acetic and β-oxy-butyric acids. The carbohydrate-free diet necessary for bringing about this result will, of course, not include bread and potatoes, but will consist entirely of such things as meat, butter, eggs, sardines and so on. Naturally, in this case, when carbohydrates are again added to the diet, the foreign acids disappear from the body.

The treatment of diabetes consists mainly in giving a considerable amount of sodium bicarbonate to neutralise the acids, and in keeping the patient thin by starvation so that he possesses a smaller amount of tissues between which to share his diminished supplies of pancreatic hormone. It is possible in many cases slowly to increase the tolerance for carbohydrates by giving gradually increasing quantities, and ensuring that the tolerance limit at any time is never exceeded. It might be thought that the simplest and most obvious treatment for diabetes would be to supply the missing substance formed by the islets of Langerhans, but so far no extract of pancreas has been obtained which contains the active principle in a

suitable form. In this respect the treatment of pancreatic deficiency stands in marked contrast to the treatment of thyroid deficiency, which yields so easily to an injection of thyroid extract. Possibly the substance concerned is too unstable.

Lastly, there is one further point which deserves attention in connection with these acids. In dealing with the fats we said that their carb n atoms were oxidised away two at a time by a process of β-oxidation. On referring to the formulæ of these acids it will be seen that there is an attempt at β-oxidation of each of them which is shewn by the presence of the $= CO$ group in the β-position in aceto-acetic acid and of the $- CHOH$ as the β-group of β-oxy-butyric acid: apparently it is in the completion of this final β-oxidation that the simultaneous oxidation of carbohydrates is essential.

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CHAPTER XII.

THE HUMAN MACHINE: ITS FUEL REQUIREMENTS AND ENERGY OUTPUT.

> "Nous sommes creés pour transformer ce que nous absorbons des choses de la terre, en une énergie particulière et d'une qualité unique."—*Maeterlinck.*

WE have dealt with the details of the chemical stages involved in the utilisation as fuels of each of the three classes of foodstuffs, but we have yet to consider the body as a whole with regard to its total fuel requirements. This is a natural and important question with regard to the working of any machine. In order to answer it we must first know the total amount of energy which the machine expends in the course of a day (including any heat loss which may occur), and also the amount of energy to be obtained from a given quantity of fuel under the conditions of working of the machine. Now energy is required by the body for the performance of muscular movements and for the maintenance of its temperature. As a matter of fact practically the whole of the energy which we expend as muscular work during our ordinary everyday activities is converted ultimately into heat. For example, the energy expended in walking or cycling along a level road is all used in overcoming frictional resistances: that exhibited by a manual worker such as a blacksmith goes to heating the material he is shaping by his vigorous blows: even the energy displayed by the muscles of my hand as I write these words is being lost as heat owing to the friction between my pen and the page

over which it moves. It is only when we use our muscles for storing up energy, say, by carrying a weight through a vertical distance, that work is done which does not immediately appear as heat. It follows from these considerations that if we could measure the rate at which heat is produced by a living creature we should know the rate at which he is expending energy, and hence know also the total quantity of combustible food he will require for use as a source of this energy. Amounts of heat energy are expressed in calories, a calorie being the amount of heat required to raise the temperature of unit mass of water one degree centigrade. For physical purposes it is often convenient to choose 1 gm. as the unit mass of water, but in the study of the energy exchanges of the body it is more convenient to take a unit a thousand times as large. So that our unit of heat energy will be the **large calorie**—the amount of heat required to raise the temperature of a kilogram of water by 1°C. It is simplest for our purposes always to express all quantities of energy in these heat units. We can do this because it has been shewn by very careful experiments that a given amount of mechanical work always corresponds to a proportionate amount of heat energy. It is the ratio of the amount of mechanical work to the amount of heat to which it corresponds which is termed the mechanical equivalent of heat. In physical experiments it is usual to measure the amount of heat given off by a body during any change in some form of calorimeter—a vessel in which the heat is measured by the rise of temperature of a known mass of water. And usually it happens that the rate of the changes investigated in such experiments is so great that it is justifiable to assume that no appreciable amount of cooling takes place during the experiment, so that the whole of the heat is

retained by the calorimeter and its contents. From an animal, however, the heat is given off slowly and continuously, and the time required for the observation is usually so long that unless the amount of heat lost by cooling

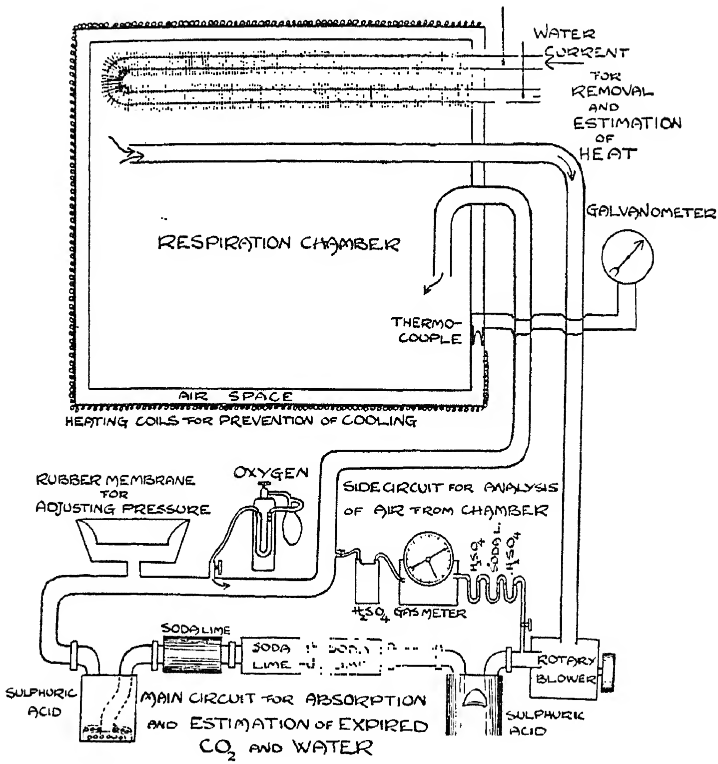

FIG. 10. A diagram of the Atwater-Benedict respiration calorimeter. (Drawn from the figures and descriptions of Benedict and Milner.)

from the calorimeter were taken into account a very erroneous estimate of the total amount of energy expended by the organism would be obtained. There are several

ways in which this difficulty has been overcome in practice. Rubner avoided it in experiments with animals by noting the rise of temperature occurring in his calorimeter in a given time, and then substituting for the animal under test a gas flame whose size was adjusted until it produced the same rate of rise of temperature as did the animal previously. He then noted the rate at which the gas was being burnt; and knowing the amount of heat liberated during the combustion of unit volume of the gas, he could calculate the rate at which the animal was producing heat, and so estimate the rate at which its oxidative metabolism was proceeding. For small animals A. V. Hill has found that ordinary Thermos vacuum flasks make good calorimeter vessels. The rate of loss of heat from these is very small, and in any case can be determined under any given experimental conditions and allowed for. But the heat insulation of a calorimeter chamber large enough to contain a man, is a problem of a totally different order of difficulty from that of producing a vessel suitable for the measurement of the heat evolved by a small animal or an isolated organ. Atwater and Benedict, working in America, have, however, succeeded in constructing such a chamber (see Fig. 10). They do not attempt to prevent loss of heat from the room containing the subject by means of insulating material, but by enclosing it in a larger chamber the inner walls of which are kept adjusted to exactly the same temperature, by means of electric heaters, as that of the outer walls of the inner chamber where the subject is. This temperature control is brought about by means of thermo-electric junctions. It is evident that, since a body loses heat only as a result of differences of temperature between itself and its surroundings, if the chamber and its surroundings are

maintained at exactly the same temperatures it will lose no heat. Or we can say that the electric heaters in the outer chamber of the Atwater-Benedict calorimeter replace the heat of the inner chamber just as fast as it is lost. The heat produced by the subject is not allowed to accumulate in the inner chamber, and so to raise its temperature: it is carried away and measured by what is called the method of "continuous flow." That is, a current of water is circulated through a system of pipes in the chamber, and is warmed by the heat evolved by the man inside. The rate at which the water flows is measured, together with the respective temperatures at which it enters and leaves the chamber. We thus know that in a noted time a certain mass of water has had its temperature raised by a certain number of centigrade degrees; we can easily calculate how many kilos of water would have had its temperature raised by one degree by this amount of heat, which is the same thing as the number of calories of heat produced by the subject in the given time.

By means of the Atwater-Benedict calorimeter it has been found that a man at rest evolves about 2,500 large calories per twenty-four hours. This is the smallest amount of energy which he must expend per day in the maintenance of the activity of his essential tissues; it is the minimum required to maintain the heart beat and the respiratory movements and to keep up the body temperature. This is referred to as his **basal metabolism.** When he performs muscular work in such a way that the whole of his energy is ultimately converted into heat—this is usually carried out by means of a stationary bicycle in which the hind wheel works against a frictional or other form of brake—the amount of heat evolved is increased in proportion to

the amount of work done, so that a man carrying out hard muscular work may give out as much as 7,000 calories per day.

In order to decide the total amount of energy-supplying food which a man requires under any given conditions of work it is necessary to know the total amount of energy to be obtained by the oxidation of a gram of each of the usual food constituents. This can be measured by burning them in an atmosphere of oxygen in a vessel, arranged so that all the heat of combustion is transferred to a known mass of water, whose rise of temperature is noted. Such an apparatus is known as a bomb calorimeter. In this way it is found that the combustion of:

1 gm. of carbohydrate	yields,	on an	average about	4.2 calories
1 gm. of fat	,,	,,	,,	9.5 calories
1 gm. of protein	,,	,,	,,	5.6 calories

But before we can use these figures for the calculation of the total energy to be obtained from a given diet of known composition we must allow for the fact that for various reasons the amount of energy to be obtained from a given amount of a material introduced into the body may be somewhat less than that evolved when it is combusted in the calorimeter. For one thing, the absorption of the digested food from the alimentary canal may not be absolutely complete, so that a certain amount of fuel will be wasted in the fæces. Then again, the oxidation of the food which has been absorbed may be less complete in the tissues than it is in the calorimeter. For example, the nitrogen of protein molecules is, as we have seen, eliminated from the body as urea. In this substance we have carbon and hydrogen which are got rid of from the body in forms other than those of their products of complete oxidation, namely, carbon dioxide and water. The

carbon and hydrogen which go to the formation of urea will, therefore, not yield their full quota of energy to the body. The physiological energy value of proteins is therefore somewhat less than the actual energy liberated during their complete combustion.

Another method of determining the quantity of fuel required by the body for the performance of a given amount of work is to determine the total amount of carbon dioxide and water produced by a well-fed man expending the given amount of energy. This is usually done by absorbing the water in strong sulphuric acid or calcium chloride, and the carbon dioxide in soda lime, while the subject breathes from a closed circuit to which oxygen is added from time to time as required. Such an arrangement is illustrated in Fig. 10 (p. 147). The amount of oxygen used by the man also can be measured by weighing the cylinder before and after the experimental period. In this way we determine the amount of carbon and hydrogen which the subject oxidises in a given time, and therefore learn the weight of these substances which must be contained in his food in order to supply his energy requirements.

While the total gaseous exchanges are being investigated in this way the subject may also be enclosed in a calorimeter such as we have already described, and so arranged that the mechanical energy he expends is all converted into and measured as heat, so that by measuring the total amount of carbon and hydrogen oxidised and the total amount of energy given out during a given time, we can compare the amount of energy which is obtained by the oxidation of these amounts of carbon and hydrogen in the body with that which would be produced if they were burnt outside the body. It is found that the two

quantities of heat are the same—in other words, that the body cannot derive more energy from a given amount of fuel than could be obtained from it by combustion in the absence of living matter. A living organism—even a human being—has, therefore, no undiscovered source of extra energy with which to supplement that contained as chemical energy in its food. He is restricted, so far as his energy exchanges are concerned, by the same laws as operate among non-living portions of matter—where energy is found never to appear spontaneously, but invariably to be derived from an equivalent quantity of energy pre-existing in some other form. Incidentally, an experiment in which the total respiratory and energy exchanges of a man are simultaneously measured shows us that if energy is not created, neither is it destroyed by living matter, so that the same total amount of heat is inevitably formed by the oxidation of a gram of carbon or of hydrogen in the human body as would be developed by the combustion of this amount of these materials outside it. In other words, none of the chemical energy is wasted during its conversion in the body into mechanical energy, and from this ultimately to heat. It is conserved just as completely as during the energy transformations in non-living matter; the law of the **conservation of energy,** therefore, applies to the living animal no less than to the dead earth on which it lives. Indeed, the law of conservation of energy was first deduced from the observation that the venous blood of the inhabitants of hot climates was of a brighter red colour, and therefore richer in oxygen than that of dwellers in colder regions. This means that less oxygen is used from the arterial blood in the case of the tropical native, because he requires less

oxidation in his tissues since he loses less heat to his surroundings, and so has less to replace than his more chilly neighbour. This suggested the idea that a given amount of oxidation must inevitably give rise to the same amount of energy, and that, therefore, the only method of controlling the heat production was the regulation of the amount of oxidation occurring.

The organism, then, for all its warm throbbing life—despite, it may be, its keen mentality, developed through long ages of painful evolutionary experience—has no power to create the minutest fraction of that energy which it requires for its continued existence; neither is it able to destroy the merest trace of the energy which it meets by contact with its environment, no matter how much this may hamper its activities; its behaviour is always circumscribed by the same inevitable laws which operate in those changes where life is not concerned.

The rate at which carbon and hydrogen are oxidised in the body depends upon two chief factors. Of these the first is the amount of energy required by the body for the production of mechanical work. Any increase in the amount of work performed by the body means, as we have seen, an exactly proportionate increase in the rate at which fuel is burnt, over and above the basal rate observed in the resting condition. Then, secondly, the total amount of oxidative metabolism occurring is determined also by the rate at which heat must be supplied in order to maintain the body temperature, and this depends on the rate at which heat escapes from the body. Now the rate of cooling will vary in proportion to the total surface of body from which heat can escape. Naturally this will be larger for a large animal such as an elephant than for a small one such as a mouse. But at the

same time it will be realised that of the total mass of matter in the body of the smaller animal a greater *fraction* is present in the surface than in the case of the larger. Relatively, much more of the bulk of the elephant's body is made up of internal structures—which are not exposed to the air, and so do not lose heat—than is the case in the mouse. This is merely an expression of the general truth that if a mass of matter—originally single—be subdivided, the surface is increased because new surfaces are formed at each place of cleavage. It is of course quite easy to imagine an elephant's carcase divided up into many small fragments, of the size of a mouse, and of these but relatively few would, if in their normal positions in the beast, abut on to the surface of the body. So that the amount of body surface in the elephant is distributed over so large a mass of material that the surface *per unit weight* is comparatively small. On the other hand, in the mouse the amount of surface per unit weight is large. This means to say that the heat loss per unit weight in the elephant is much smaller—other things being equal—than in the mouse. We should therefore expect that the amount of fuel oxidised per unit weight in the case of an elephant is much less than that per unit weight of mouse. And such is indeed the case. But when we calculate the **total metabolism per unit area of surface** we find that it is approximately constant for all species and independent of their various sizes.

The total respiratory exchange is also dependent in a certain way upon the diet. Not that the combustion in the tissues depends on the amount of fuel supplied to them—for the contrary is the case. Excess of fuel is not burned wastefully, but is stored in the body. But the rate of combustion is influenced by the composition

of the food—and particularly by the proportion of proteins it contains. For it is found when abundant protein is fed, that the presence of this, or rather of its breakdown products, in the tissues, leads to a marked increase of the rate of total metabolism even in a resting subject. This acceleration of the metabolism is referred to as the **specific dynamic action** of proteins. At present we are uncertain as to the reason for this behaviour. It may be that the de-amination of the amino-acids requires the expenditure of energy in the tissues where this process goes forward, or it may be that either the amino-acids themselves, or internal secretions formed from them, exert a direct stimulating influence on cell metabolism, and cause the process of oxidation of fats and carbohydrates to proceed more vigorously. But whichever explanation comes to be accepted finally, the observation that in the presence of excess of amino-acids the combustion of *other* constituents of the cell protoplasm is accelerated, is well-founded. The extra energy of this more rapid oxidation, not being required for the performance of mechanical work, is lost from the body as heat.

It is this stimulating effect of proteins on the oxidative metabolism which probably accounts for the fact that these substances do not become converted into fat in the body, although there is no reason for supposing that any of the necessary steps in this conversion are chemically impossible (p. 102). Rather it seems that when protein is eaten in sufficient amount to leave an excess which might be stored as fat, the oxidation of materials is so stimulated that any fat which might otherwise have been formed and stored, is at once burnt up to carbon dioxide and water.

The study of the total respiratory exchange, that is, of the amount of oxygen taken into the body, and of carbon

dioxide given out per minute, gives us information not only as to the rate at which carbon is being oxidised, but also as to the actual class of foodstuff which is undergoing combustion at any moment. For, of a certain amount of oxygen used in the body, some will go to the oxidation of the carbon, and the rest to the oxidation of the hydrogen of the fuel. And, of course, the proportion of the amounts of oxygen used for these two purposes is determined by the ratio of the numbers of atoms of carbon and hydrogen in the substance undergoing oxidation. We can quite easily determine the ratio of the amounts of oxygen used for oxidising carbon and hydrogen respectively by measuring the amounts of carbon dioxide and of water produced by the body in a given time. This is particularly easy in the case of the carbon dioxide, because there is but one channel for the excretion of this substance—namely, the expired air. Indeed, in order to discover the fraction of the oxygen used for oxidising carbon we need to observe simply the total amount of oxygen used per minute, and the amount of carbon dioxide produced during the same time. Since any given quantity of carbon dioxide contains exactly its own volume of oxygen, the volume of carbon dioxide produced is equal to the volume of oxygen used to oxidise the carbon, so that the ratio

$$\frac{\text{volume of carbon dioxide produced}}{\text{total volume of oxygen used}}$$

will tell us the proportion of the total amount of absorbed oxygen which is being used for oxidising carbon. This ratio is termed the **respiratory quotient.** Let us illustrate by means of examples the way in which a knowledge of the value of this quantity enables one to deduce the kind of material which is undergoing oxidation in the

body at any moment. Consider first of all the case of glucose. Whatever the intermediate stages, its ultimate oxidation to carbon dioxide and water may be represented by the equation:

$$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O.$$

From this it will be seen that for each molecule of oxygen used up, a molecule of carbon dioxide is formed; therefore, for each cubic centimetre of oxygen utilised, a cubic centimetre of carbon dioxide is evolved. In other words, since the glucose molecule already contains enough oxygen to account for the oxidation of its hydrogen atoms to water, the amount of oxygen which must be supplied from outside, in order to oxidise glucose completely, is equal to that which would be required for combination with the carbon. But of course it must not be supposed that the actual oxygen atoms originally contained in the glucose molecule appear exclusively as water, and that none of them go to form carbon dioxide. We have represented the relationships in this way merely for convenience in estimating the amount of extra oxygen required for the complete oxidation of the sugar. And seeing, as we have said, that the volume of oxygen required for the production of a given volume of carbon dioxide is equal to that of the carbon dioxide itself, it follows that if glucose—or, indeed, any other carbohydrate—were the only fuel undergoing oxidation in the body at any moment, the respiratory quotient would be equal to 1.

Next, let us consider the case of fats, as represented by tri-stearin (p. 96). The total formula obtained by adding up all the atoms of carbon, hydrogen and oxygen atoms in

the molecule of this substance is found to be $C_{57}H_{110}O_6$, so that its ultimate oxidation is to be represented thus:—

$$C_{57}H_{110}O_6 + 81 \cdot 5O_2 = 57CO_2 + 55H_2O.$$

In this case we observe, first, that the fuel contains very little oxygen in itself; the molecules of the fats are composed almost entirely of carbon and hydrogen. It is for this reason that a gram of fat gives out more energy on combustion than the same weight of carbohydrate or protein; the fat is composed almost entirely of combustible material, whereas the carbohydrate contains large quantities of oxygen, and the protein of nitrogen. Further, on account of the relatively small proportion of oxygen contained in the molecule, there is much less than would be necessary to oxidise more than a small fraction of the carbon and hydrogen atoms of the molecule; oxygen has therefore to be supplied in sufficient amount to oxidise practically the whole of the carbon and hydrogen of the molecule. So that if fat alone were being oxidised in the body, only a certain fraction of the total quantity of oxygen absorbed by the body would appear in the form of carbon dioxide in the expired air, the remainder going to form water. The ratio of the total volume of oxygen absorbed to that of carbon dioxide evolved would, therefore, be less than 1. We can calculate, from the equation just given, what the exact value of the respiratory quotient in the case of the combustion of tri-stearin would be. For of every 81·5 volumes of oxygen used for the oxidation, 57 volumes appear finally as carbon dioxide; the respiratory quotient, if tri-stearin alone were being oxidised in the body, would therefore be $\frac{57}{81 \cdot 5}$, which is almost exactly ·7.

From knowledge of the average composition of

proteins, we deduce, by an exactly similar line of reasoning, that the respiratory quotient during the oxidation of protein alone would be ·8. This value is intermediate between that for pure carbohydrate and pure fat, and so would also be observed during the oxidation of an appropriate mixture of these two non-nitrogenous fuel substances; but the two cases can readily be distinguished by noting the rate of output of nitrogen in the urine, for this serves as a measure of the rate at which protein is being broken down.

A knowledge of the total respiratory exchange will enable us, then, to calculate the total quantity of carbon and hydrogen undergoing oxidation in the body; the total quantity of nitrogen in the urine will tell us how much of this carbon and hydrogen is derived from proteins; and a determination of the respiratory quotient will make it possible to apportion the remaining carbon and hydrogen between the carbohydrates and fats. In this way we can decide exactly how much of each of these three substances is being oxidised in the body at any moment.

It is evident that such a complete knowledge of the combustion processes occurring in the tissues must be of first-rate importance in the study of the working of the body as an energy machine. For example, the simple observation that during muscular exercise the respiratory quotient rises slightly above its resting value shows that while, on the whole, the same materials are being oxidised as during rest, yet there is a tendency for stored carbohydrates to be utilised in somewhat greater proportion than other materials. This conclusion is important because it was at one time supposed that protein alone was capable of supplying mechanical energy when oxidised in the muscles, and that the oxidation

of fats and carbodydrates merely served to maintain the temperature of the body. This was Liebig's view. It remained prevalent only until accurate methods for the estimation of nitrogen in urine became elaborated. Then it was found that the performance of a measured amount of work was accompanied by the oxidation of much more material than corresponded with the amount of protein which could have been required to furnish the observed total amount of nitrogen excreted during the work. The classical experiment on this subject was performed by Fick and Wislicenus, who measured their respective outputs of nitrogen during an ascent of the Faulhorn. They calculated the amount of protein to which this nitrogen corresponded, knowing the average percentage of nitrogen in proteins. For Wislicenus this came to be 37 grms. We already know that the maximum amount of energy to be obtained by the complete oxidation of 1 grm. of protein is 5·6 calories, and since each calorie is equivalent to 425 kilogram-metres of work, the total amount of energy which Wislicenus could obtain for the oxidation of his protein was $37 \times 5{\cdot}6 \times 425 = 92{,}310$ kilogram-metres. Now Wislicenus himself weighed 76 kilo., and during the climb he pulled himself through a height of 1956 metres. He therefore did 76×1956, i.e. 148,656 kilogram-metres of work. It is evident that, even assuming, as we have done, that the whole of the energy of oxidation of the proteins was converted into mechanical work, only a fraction of the total energy expended in the climb can be accounted for; and when it is realised that of the total energy liberated on the oxidation of any foodstuff by far the greater amount is lost as heat, it will be seen how comparatively small was the share of mechanical energy supplied by the oxidation of proteins in Fick and

Wislicenus' experiment, and how considerable was that derived from non-nitrogenous materials. Indeed, now-a-days, we regard carbohydrates and fats as the chief fuels used by the body for the obtaining of mechanical energy, and believe that the carbon and hydrogen of protein molecules are used in this way only in case of emergency, or when proteins are present in excess in the food.

To return to the question of the respiratory quotient—not only does the value of this ratio give us information as to the substances being consumed in the normal body, but it also undergoes changes from the normal value when the course of metabolism becomes deranged. For example, the failure of the oxidation of carbohydrates during diabetes, results, as would be expected, in a low value of the quotient, corresponding to a preponderance of the oxidation of fats and proteins. And this is exaggerated by the circumstance that, in this pathological condition, there occurs an increased conversion of amino-acids into sugar through the keto-acid stage (p. 141). This change involves an oxidation, since the carbohydrate contains a relatively greater percentage of oxygen than is present in amino-acids, but the sugar, when formed, is not further burnt, but escapes in the urine. The oxygen which is taken up during this oxidation therefore does not appear as carbon dioxide in the expired air, so that the ratio $\frac{CO_2}{O_2}$ is still further reduced. The excretion of aceto-acetic and β-oxybutyric acids—the highly oxygenated products of the partial oxidation of fats—also tends in the same direction.

Some interesting changes in the respiratory quotient are observed in connection with the hibernation of animals. In preparation for hibernation a marmot, for

example, lays down an abundant store of fat, which is formed from the carbohydrate of its food. Since carbohydrate contains a greater proportion of oxygen than fat, it follows that during the change from carbohydrate to fat a certain amount of this oxygen must become available for oxidations in the tissues. Less oxygen is therefore required to be absorbed from the inspired air, so that the value of the ratio $\frac{CO_2}{O_2}$ tends to be large, and indeed may exceed 1. On the other hand, during the actual period of hibernation the stored fat becomes gradually reconverted into carbohydrates—chiefly glycogen. This change requires the addition of oxygen atoms to the constituents of the fat molecules, so that in this case an extra amount of oxygen must be absorbed from the inspired air. The value of the ratio $\frac{CO_2}{O_2}$ therefore tends to be diminished, and, indeed, it has been observed to fall as low as ·3, because the carbohydrate is not oxidised as rapidly as it is formed, so that the carbon remains stored, although no longer as fat.

The combination of oxygen in this way causes the animal to increase appreciably in weight, although while hibernating it takes no food. Of course a certain quantity of material is slowly oxidised completely to carbon dioxide, for the maintenance of such essential functions as the heart-beat—but the amount so used is small, particularly as a hibernating animal loses but little energy in the form of heat: for it no longer maintains a constant high body temperature as it does in the active condition, but becomes virtually cold-blooded—taking on the varying temperature of its surroundings.

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CHAPTER XIII.

ENZYMES AND THEIR ACTIVITIES.

"A little leaven leaveneth the whole lump."—*St. Paul.*

THE student must have noticed that at many points in our account of chemical changes occurring in the body we have mentioned that the particular change in question has been brought about under the influence of a ferment or enzyme. But up to the present we have said nothing: about enzymes themselves, or of the general characteristics of their activities. To this task we address ourselves in the present chapter. That definite chemical changes are associated with particular living organisms has long been known: for example, the power of yeast to convert sugar into alcohol and carbon dioxide is said to have been discovered by Noah, if not by one of his predecessors; and the discovery has been utilised since those remote ages, on the one hand for the production of gas wherewith to bring about the "rising" or leavening of the bread of man's food, and on the other as the means of producing that wine with which his heart is gladdened. It is hardly surprising that such processes should come to be considered to be dependent on the presence and properties of living matter itself. Liebig held the view that the changes brought about by micro-organisms were due to molecular vibrations which were set up by the living protoplasm, and which were of sufficient strength to shatter the molecules of non-living materials which were in its

vicinity. Pasteur showed conclusively that fermentations and bacterial decompositions would not occur in media in which all living organisms had been killed by heat. But a new turn was given to the subject when Buchner in 1896 showed that alcoholic fermentation of sugar took place not only in a solution in which there were present actual living yeast cells, but also in one to which the juice of the cells had been added. This juice was obtained by grinding up the cells with sand in order to liberate their contents and then submitting the mass to pressure in order to express the liquid. Careful tests were made to ensure that this was really free from living yeast cells; and the observation is now a well-established one, having been often repeated since Buchner first described it.

The process of fermentation therefore does not require the co-operation of the living yeast plant; it suffices that some particular constituent of its protoplasm should be present. To this substance Buchner gave the name **zymase** (Gr. *ζυμη*—yeast). This zymase is not part of the living matter, but is a kind of reagent which the yeast cell produces and uses in order to bring about these chemical changes which it requires for its metabolism. We now realise that most, if not all, of the chemical reactions occurring in living cells are brought about by reagents, similar to the zymase of yeast. These are collectively termed **enzymes** or **ferments.** Throughout the previous chapters of this book we have mentioned a number of such enzymes, and have described the changes which are brought about under their influence. It will have been noticed that in some cases these enzymes act in the cells themselves; this being the case with those which are concerned with tissue metabolism; on the other

hand, the enzymes which bring about digestive changes are elaborated by the glands of the alimentary canal, and are poured out to act outside the cells which produced them.

A moment ago we referred to enzymes as being the reagents used by living tissues. Rather we should more exactly have compared them with the catalysts with which the non-biological chemist is familiar. For while a reagent is usually used up during a reaction—being one of the reacting substances—it is of the essential nature of a catalyst that it remains unchanged in amount in the system in which it has influenced the chemical changes, so that, apart from secondary circumstances, a very small amount of the catalyst will serve to produce change in a very large—almost indefinite—amount of the substances submitted to its activity. The same is true also of enzymes. They are catalysts elaborated by living matter for influencing the chemical changes on which the continued existence of that matter depends. Now seeing that a catalyst remains unchanged in the system when the particular chemical change has taken place, it is evident that it cannot have added any energy to the system. Energy could only be obtained if the catalyst underwent some chemical change—for example, an oxidation—whereas, as a matter of fact, observation shows that it remains unaltered.

It therefore follows that the only respect in which an action catalysed by an enzyme differs from that occurring between the same initial substances when left to themselves will be that of velocity. The substances if left to themselves will produce exactly the same equilibrium mixture as in the presence of the enzyme, only much more, often infinitely, slowly. For a change of equilibrium

would necessitate a change in the energy content of the system; in order that the reaction might proceed further, for example it might be that extra energy would need to be supplied. But of furnishing this extra supply of energy a catalyst is incapable, because at the end it retains all the energy it possessed at the beginning of the reaction. This fact has been proved in detail in the case of the hydrolysis of an ester by means of a fat-splitting enzyme, lipase—a convenient case to deal with experimentally, as the action even in the absence of an enzyme proceeds at a rate which is sufficiently rapid to be observed with reasonable ease. Suppose, for example, one takes an ester such as ethyl acetate, and leaves it in contact with water for some weeks or months, then it is found that the substance is slowly hydrolysed with the formation of acetic acid and ethyl alcohol:—

$$CH_3.COOC_2H_5 + H_2O \rightleftarrows CH_3COOH + C_2H_5OH.$$

The reaction proceeds slowly until a mixture in certain proportions of ethyl acetate, water, acid and alcohol has resulted. No further change then takes place, and the system remains permanently at this equilibrium composition. The reason for this behaviour is found in the fact that in such a system we really have two reverse reactions proceeding at each moment. The one is the hydrolysis of the ester to liberate the free acid and the alcohol:—

$$CH_3.COOC_2H_5 + H_2O \longrightarrow CH_3.COOH + C_2H_5OH.$$

The other simultaneous reaction is the reverse of this and consists of the combination of the acid and alcohol to give the ester and water again:—

$$CH_3.COOH + C_2H_5OH \longleftarrow CH_3.COOC_2H_5 + H_2O.$$

Now the rate at which each of these reactions proceeds is

determined by the concentrations of the reacting substances. At the commencement the concentrations of water and of ester are relatively high, and the hydrolysis proceeds quickly. But as this hydrolysis proceeds, ester and water are being gradually used up so that their concentrations are falling off, and the rate at which the change proceeds becomes progressively slower. But the rate of the re-combination of acid and alcohol, slow at first, is meanwhile gaining in speed, for the concentrations of these substances increase as they are liberated from the ester. The rate of the forward change, then gradually slows and that of the backward reaction gradually increases until there comes a moment when the rates of the two are just equal. At this point equilibrium is attained, and no further change appears to take place because the ester is breaking up just as fast as it is being re-formed. If now we start with the same concentrations of initial substances, but add to the mixture also a catalyst such as a little dilute acid, or an ester-splitting enzyme, then exactly the same kind of result is observed. The same equilibrium mixture is obtained, but in this case much more quickly. This being so, it follows that not only has the catalyst added no energy to the system, but also that it must have accelerated the backward and forward reactions to exactly the same extent, for the final equilibrium condition arrived at depends on the *ratio* of the actual backward and forward velocities, and it is only if these are increased each in the same proportion that the ratio remains unaltered. From a slightly different point of view this is equivalent to saying that no matter whether we start with a mixture of ester and water, or of acid and alcohol, we shall ultimately obtain the same final mixture of all four substances; both in the presence

of the catalyst, when the equilibrium will be attained rapidly; and in the absence of the catalyst, when the same result will be achieved much more slowly. This has been shown to be true in the case of the fat-splitting enzyme lipase, and is now generally held to apply to all enzymes.

Since enzymes, then, accelerate both the forward and the backward reactions in a chemical system which is under their influence, they are capable of bringing about not only breaking-down changes, but also the reverse processes of synthesis. This we have illustrated repeatedly already. We might remind the student by way of example of the activities of the proteolytic enzymes of the alimentary canal. These bring about the hydrolysis of proteins into amino-acids. The tissues also contain proteolytic enzymes, but these assist in the building up into tissue proteins of the amino-acids absorbed from the alimentary canal. The direction in which a change occurs in a chemical system depends entirely upon the relative concentrations of the reacting molecules. If any of these be present in large amount, then, by force of numbers, so to speak, they insist on having a share in the chemical reaction, and so on being used up. On the other hand, if any kind of molecule is present only in small concentration, then there is plenty of room for the production of more of it, so that in general the reaction will tend to proceed in such a way that this result is brought about. A catalyst will not influence the direction in which the change tends to occur, it will merely accelerate the change when it does occur. In this respect the catalyst behaves exactly as does the oil on a machine; it accelerates the movement, but does not initiate it or determine its direction.

We must now go on to consider the various kinds of

reactions which are influenced by enzymes. First, of course, there are the breakdown changes occurring during digestion. The student has already learned how starch, for example, is converted under the influence of the ptyalin of the saliva into maltose, how the proteins of the food are broken down first by the pepsin of the gastric juice into peptones, and then further into their constituent amino-acids under the influence of trypsin and erepsin. The splitting of fats into their fatty acids and glycerine has been already used as an example in this present chapter. Then previously (p. 49) we have mentioned the enzyme urease which is used to convert urea into ammonia in the modern method of urine analysis. It will be noticed that all of these changes are processes of hydrolysis, and indeed a large number of the reactions brought about under the influence of enzymes are of this kind; but not all. There are two important groups of enzymes which control changes of other kinds. Of these groups one includes those enzymes which are responsible for processes of clotting—the **rennin** of the gastric juice of young mammals, which clots milk, and the **thrombin** which is concerned in the clotting of blood—while the other group includes those enzymes which are concerned with processes of oxidation in tissues. As these last-named enzymes are of a rather special nature, we shall postpone our account of them until we have dealt with the more typical characteristics of enzymes in general.

Now, although, in so many cases, the reactions brought about under the influence of enzymes are processes of hydrolysis it must not be supposed that any given enzyme is capable of accelerating all such processes. For there is a marked specificity on the part of enzymes for the substances on which they will act. Pepsin will only split up

protein molecules, and ptyalin only starch; although in each case the change is, as we have seen, one of hydrolysis. In the case of the digestive enzymes, usually each enzyme will act upon a whole group of substances of similar chemical constitution, but in other cases their degree of specificity is extreme. For example, some enzymes will distinguish between one optical isomer of a substance and the other, attacking the one, but leaving the other quite unaffected. This has suggested the simile that a enzyme needs to be adapted to the substance it is to act upon—to its **substrate** as it is called—just as a key must be adapted to a lock which it is to open.

We might at this point refer to the nomenclature which has been adopted for enzymes. Seeing that the chemical constitution of no enzyme is at present known it is impossible to give systematic chemical names to these substances; under these circumstances it has been found most convenient to name each enzyme, or, in many cases, each type of enzyme after the substance on which it exerts its action. The termination "-ase" is usually added to the name of the substrate in order to form the name of the enzyme. Thus, an enzyme which hydrolyses starch is called an amylase (Latin, amylum = starch); a fat-splitting enzyme is known as a lipase (Gr. λιπος = fat). In order, then, to refer to any particular enzyme, its usual place of occurrence is usually also mentioned. Thus one speaks of the pancreatic lipase, and so on. But this method of naming ferments has not been applied to a few which are already known before the present nomenclature was introduced. For example, enzymes which attack proteins are still called proteolytic enzymes, and they are usually referred to by names which do not conform to the above rule. Pepsin and trypsin have become such household

words in the language of the biochemist that one would be reluctant to replace them even were it desirable to do so.

By this time the reader is doubtless wondering what an enzyme really is, and how it comes to possess such useful properties as those we have been mentioning. The answer to these questions is only incomplete at present, partly because so far no enzyme has been isolated in the pure condition. In general, we can only deduce the presence of an enzyme from the occurrence of the reactions which it brings about. But nevertheless, enzymes can be prepared, not only in solutions derived from living tissues but, also in a solid but impure condition by various processes of precipitation. The zymase of yeast can be so obtained, and so also can the glycogenase which occurs in the liver. But the difficulty is that the acetone or alcohol used to precipitate the enzyme throws out of solution also the proteins which accompany it in the tissue fluid. However, we have evidence that enzymes are themselves proteins. It is known, for example, that a particular enzyme can be digested by another proteolytic enzyme. Then, also, like proteins, enzymes are particularly sensitive to heat. If a reacting mixture, in which enzyme action is occurring, is warmed, the rate at which the reaction proceeds is increased; this, of course, is the usual effect of rise of temperature in any chemical reaction. But above a certain optimum temperature at which the reaction is most rapid, it begins to fall off in rate again as the temperature is still further raised. This is because at the higher temperature the general increase in reaction velocity is accompanied by a partial destruction of the ferment. So that while the remaining portion is working more rapidly than ever, the amount of enzyme present is so much

diminished that the total aggregate rate of change is slowed.

The manner in which an enzyme works is undoubtedly very complicated, but it is certain that one step in the process consists in the combination of the enzyme with its substrate. This combination may be a physical process of surface concentration of the molecules of the substrate and to the colloidal enzyme particles—a phenomenon to which the name **adsorption** is given. In this case the accelerated decomposition of substrate might be partly explained on the grounds of its increased concentration. Or the combination between enzyme and substrate may be of a more strictly chemical nature, in which case one would suppose that the combination between the two and the breaking up of the resulting complex form two successive chemical reactions which together take much less time than the more direct unaided breakdown of the substrate alone. Cases of this kind are not unknown among inorganic catalysts. If we suppose that the initial combination between enzyme and substrate takes place almost instantaneously, then we can easily understand the results of experiments in which the relation between the rate of an enzyme action and the relative amount of enzyme to substrate has been investigated. Take, for example, the case where an enzyme is influencing a process of hydrolysis. It is evident, of course, that, as the action proceeds, the concentration of the substrate gradually falls off, for it is being decomposed. But the rate at which the decomposition occurs does not fall off to a corresponding extent: indeed, the rate of the reaction is found to remain unaffected for a long period, in spite of the reduction of the concentration of the reacting substrate. This forms a marked contrast to the behaviour

observed in the case of a reaction which is not being catalysed by an enzyme; here the rate of the reaction begins to slow down at once as soon as the concentrations of the reacting substances are at all diminished. From this observation it is argued that the enzyme and the substrate rapidly enter into some kind of union, and that it is only that portion of the substrate which is thus combined with enzyme which undergoes further chemical

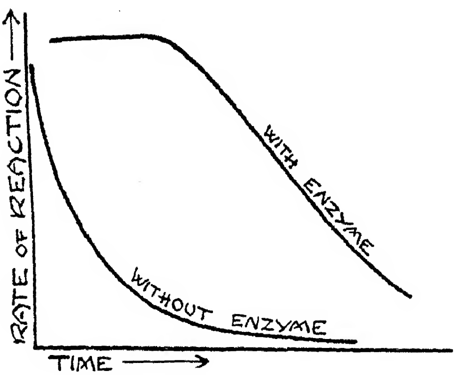

FIG. 11. A diagram showing how the rate of many enzyme actions remains constant until there remains too little of the substrate to "saturate" the enzyme.
The uncatalysed reaction becomes slower and slower from the commencement as the reacting substances become used up.

change at an appreciable rate. Now as long as there is a sufficient excess of the substrate the enzyme will be completely "saturated" with it, for any of the combined substrate which becomes decomposed will be replaced from the free excess substrate in the solution. Thus the concentration of combined substrate, i.e. of substrate undergoing decomposition, is maintained constant over a long period of time, so that the rate at which the decomposition occurs is also constant. Naturally, so soon as so much substrate has been removed by decomposition that there no longer remains enough to "saturate" the whole of the enzyme present, then the concentration of the enzyme-substrate compound falls off, and with it

the rate of the decomposition. These relationships are dealt with diagrammatically in fig. 11.

We have already referred to the influence of temperature on enzyme action. It is necessary to point out that temperature is not by any means the only factor which affects the rate at which an enzyme works. The changes in the nature of the medium, variations in the kind and amount of salts present, for example, or in the degree of alkalinity or acidity of the fluid, exert very powerful effects on the activity of enzymes. The ptyalin of saliva acts very slowly on starch in the complete absence of sodium chloride, and its action is completely arrested if the mixture be made acid. On the other hand, pepsin will attack the protein molecule only in an acid solution, while trypsin requires an alkaline medium for the manifestation of its somewhat similar activity.

In general we may say that there is an optimum reaction for each particular enzyme at which its efficiency is a maximum. It is on account of its sensitiveness to changes in environment that an enzyme is often able to bring about change in a limited quantity only of its substrate, for in many cases the products liberated during the activity of the enzyme are such as inhibit its continued action. So that unless these products are removed or neutralised, the action comes to rest. This is more particularly the case when one product of the decomposition is an acid, as for example during the fermentation of milk sugar to lactic acid. In this case if a good yield of the acid is desired it is essential to have excess of calcium carbonate or zinc carbonate present in order to neutralise the acid as it is formed.

Then again, not only must an enzyme be provided with a suitable medium in which to act, but often also it

requires to be " activated " before it will bring about any changes at all. In other words, an enzyme does not usually occur in the active condition in the secretion of the gland which produces it. More often it is turned out from the gland in the form of an inactive precursor which requires to meet some other activating substances before it is converted into the free potent enzyme itself. A case we have already met is that of trypsin. This proteolytic enzyme is not itself present in the pancreatic juice, it is represented by its precursor trypsinogen from which trypsin itself is only obtained when the precursor meets the activator enterokinase present in the intestinal juice. For this reason pancreatic juice, which is collected straight from a pancreatic duct without contact with the tissues of the intestine, is found to be devoid of action on proteins. This seems to constitute a mechanism whereby the tissues of the pancreas, and for that matter probably also the other constituents of the pancreatic juice, are protected against the powerful digestive action of the trypsin.

With regard to the way in which the enterokinase works it seems that in this particular case, at all events, we are dealing with an activator which is itself an enzyme, for trypsinogen seems to be a complex of trypsin together with a protein molecule, which latter it is the function of the kinase to remove.

A very similar state of affairs is found to exist with regard to thrombin, the enzyme or enzyme-like substance, chiefly responsible for the clotting of blood. Briefly we may say that while the substrate fibrinogen, from which the essential constituent of the clot, fibrin, is formed during the act of clotting, is present in the circulating blood, thrombin is only present in the form of its

precursor prothrombin. This prothrombin is converted into thrombin only when it meets the activator thrombokinase, which is liberated from the disintegrating platelets and damaged tissues when the blood is shed.

It is interesting to note that both entero- and thromkinase require the presence of a certain concentration of calcium ions for their activity; indeed, many of the commoner methods for preventing the clotting of blood, such as the addition of oxalate, citrate or fluoride, owe their efficiency to the fact that they either precipitate the calcium in an insoluble form, or, as in the case of the citrate, they convert it into a form which though soluble, does not give rise to calcium ions.

Not only do we know of substances which assist the action of ferments, but we know also of others which exert an antagonistic action. For example, the stomach wall protects itself against the digestive action of pepsin by the secretion of an anti-pepsin which hinders the action of the ferment on the protein of the tissues. After death the tissues no longer secrete anti-pepsin, and so become liable to post-mortem digestion. Then again, the blood is kept from clotting in the vessels by means of an anti-thrombin—a substance which prevents the action of such small quantities of thrombin as may from time to time be formed. It is interesting to find that the substance which the leech injects in order to prevent clotting of the blood on which it feeds is an anti-kinase. It does not prevent the action of thrombin when this is once formed, but hinders the formation of the thrombin from its precursor.

We have yet to deal with the enzymes which accelerate processes of oxidation in tissues. These are by no means the least important of the changes occurring in the body

which require to be accelerated by enzymes, for on them the energy supply to the tissues depends. Now the substances which are oxidised in the tissues are not as a rule liable to oxidation if merely exposed to gaseous oxygen, or, for that matter, to oxygen in solution. If these substances then, are to be oxidised with measureable speed some enzyme must be present which will accelerate the reaction suitably. Such substances have been isolated from tissues and have been given the name **oxidases.** To their activity is ascribed not only the normal oxidations occurring in the cell, but also such oxidations as are evidenced by the visible colour change in a cut apple or potato on exposure to air.

It has been found, however, that the matter is not quite so simple a one as we have just described. For the substances which have been termed oxidases have proved not to be just simple enzymes accelerating the reaction between molecules of oxygen and those, say, of glucose. On the other hand, the oxidases have turned out to be complexes containing what are known as **autoxidisable** substances. These are substances which are usually of the nature of complex organic peroxides. These are formed by the direct union of molecular oxygen with a complex organic molecule. They are not stable, however, and readily break down again giving nascent oxygen, i.e. oxygen in the form of single atoms, and the original organic complex which is available for forming a fresh quantity of peroxide with a further supply of oxygen molecules. The oxygen atoms which are liberated from the peroxide in the way we have described are much more vigorous oxidising agents than ordinary oxygen molecules. By this roundabout process of peroxide formation we have thus obtained the oxygen in a more active form. And

we find that, lest the whole series of changes should mean delay, the decomposition of the peroxide is accelerated by a further enzyme termed a **peroxidase.** So that this oxidase system comes to consist of two constituents—autoxidisable substance capable of forming a peroxide with **molecular** oxygen and a peroxidase enzyme which accelerates the decomposition of this peroxide, thus leading to the liberation of **atomic** oxygen, which forms the primary oxidising agent of the cell.

Lastly, we might just mention the question of the detection and estimation of enzymes. Although we do not know the chemical constitution of any enzyme, and so cannot test for it or estimate it as a chemical substance, yet we achieve both of these results by taking advantage of its own characteristic activity. For example, if we wish to test for a starch-splitting enzyme in a solution we add a little starch paste to the solution, and, taking care that the reaction is about neutral, keep the mixture in a warm bath at body temperature. If then the mixture gradually loses the property of giving a blue colour when a drop of it is treated with iodine, we conclude that an amylase is present in our original solution. And we confirm this conclusion by determining that boiling a portion of our solution leads to a destruction of its starch-splitting power—thus proving that the action was due to an enzyme, since, as we have seen, enzymes are destroyed at a boiling temperature. Similarly in testing for a proteolytic enzyme we investigate whether our given fluid will cause the hydrolysis of a suitable protein added to it. In the case of pepsin it is usual to use fibrin (from a blood clot) stained with carmine. The mixture must, of course, be made acid with ·04 per cent. hydrochloric acid. If then the fibrin becomes hydrolysed the carmine

is liberated into the main bulk of the liquid. As before, a control must be carried out with some of the fluid which has been previously boiled in order to prove that the liberation of the carmine was actually due to an enzyme action. The hydrochloric acid itself will not extract the dye, but alkali will do so, so that the stained fibrin cannot be used in testing for trypsin since for this a medium made alkaline with sodium carbonate is required. In this case it is convenient to employ a solution of some protein which is soluble in alkaline, but not in acid solutions. Casein is a case in point. Then, as the hydrolysis proceeds, the casein is broken down into amino-acids which are soluble not only in alkaline, but also in acid solutions; so that, as the casein disappears, less and less precipitate is produced when a test portion of the reacting mixture is acidified: the disappearance of the casein then being a proof of the presence of a proteolytic enzyme which acts in an alkaline medium. Of course, as usual, this must be confirmed by proving that this proteolytic activity of the solution is destroyed on boiling. It is evident that in order that the test shall be carried out conveniently only a small quantity of casein must be added. Otherwise it may be that the time required for an appreciable reduction in the amount of unchanged protein precipitated on acidification will be excessively prolonged. In a similar way it is necessary to avoid the addition of too much starch in testing for amylase, and, in general, in testing for enzymes, only to use an appropriate small quantity of substrate.

The estimation of the degree of enzyme activity is carried out in a similar fashion. Either the amount of substrate acted upon under standard conditions in a given time or the time taken for the complete digestion

of a given amount of substrate is measured. For example, we can compare the activities of two pepsin preparations by placing in each glass capillary tubes filled with coagulated egg-white, and noting the distance to which the digestion has advanced along the several tubes after the lapse of a known interval of time. The activities of trypsin solutions, on the other hand, are estimated by determining the length of time required for the complete digestion of a known amount of casein or other suitable protein.

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The Oxidases and other Oxygen-Catalysts concerned in Biological Oxidations, by J. H. KASTLE. (Bulletin No. 59 from the Hygiene Laboratory of U.S. Public Health and Mar. Hosp. Service, Washington.)

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Physiological Oxidations. (*Physiological Reviews*, Vol. I. (1921), p. 394.)

CHAPTER XIV.

ACCESSORY FOOD SUBSTANCES OR VITAMINES.

"We came to few places where either the art of man or nature did not provide some sort of refreshment or other, either of the animal or vegetable kind. It was my first care to procure what could be met with of either by every means in my power, and to oblige our people to make use thereof, both by my example and authority; but the benefits arising from such refreshments became so obvious that I had little occasion to employ either the one or the other."—*Captain Cook.*

Ever since the time when shipping first became sufficiently developed to enable long voyages to be taken out of sight of land it has been a matter of common experience that a diet composed of entirely preserved foodstuffs is not adequate for maintaining the members of a crew in health over long periods of time, although the preserved food may contain sufficient fuel substances to supply the energy needs of the body, and suitable proteins for repairing its wear and tear. The commonest disorder which is liable to appear under these conditions is the disease known as **scurvy,** in which the chief characteristic is the occurrence of numerous small hæmorrhages throughout the body, more particularly between the teeth and round the bones and joints, the end effect being almost complete loss of muscular power in the limbs. At first these symptoms were ascribed to the excess of salt used to preserve the food, but now it is realised that they are due to the absence from the preserved rations of small quantities of some substance which is essential for the maintenance of health, and which, though originally

present in the fresh foods, is so readily decomposed as to be lost during the processes of preservation. That this is the correct explanation is shown by the fact that the missing substance can be supplied in sufficient quantity if small amounts of fresh fruit or vegetables are added to the dietary. It is found that the necessary substance is contained in suitable quantities in lime juice, and that it can be stored in this form for a long time. Hence the reason why expeditions to the arctic and antarctic regions, and similar desolate wastes, are always abundantly supplied with lime juice in order to prevent the outbreak of scurvy among the members of the exploring party.

Such substances, which are necessary for continued health, and are present in fresh foods, but are destroyed by the processes of cooking and preserving, are termed **accessory food substances** or **vitamines,** the second name expressing the disproven supposition that these substances, so essential for the normal carrying on of vital processes, are related chemically to the amines. The particular one which is necessary for the prevention of scurvy is called the anti-scorbutic vitamine, and also **water-soluble C,** a name which indicates that it is present in aqueous plant and fruit juices, and which furnishes a suitable designation until we can replace it by a more illuminating chemical appellation. Another vitamine, of importance equal to that of the anti-scorbutic substance, is found to be associated with the fat of natural foods, and for this reason is usually called **fat-soluble A.** It has two chief functions: in the first place it is essential for the growth of a young animal. It is found that if young rats are fed on a diet composed of protein, starch, lactose and inorganic salts, all of which have been previously extracted with alcohol to remove the vitamine, the

animals cease to grow after a week or two and then gradually decline in weight and die after about six weeks. On the other hand rats which receive the same basal diet together with 2 c.c. of fresh milk a day will grow normally and thrive. Furthermore rats which are declining in weight on the artificial diet will rapidly make up the deficiency and then proceed along a normal course of

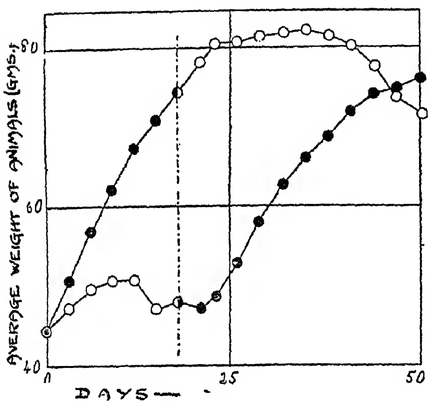

FIG. 12. To illustrate the influence of the vitamines in fresh milk on the growth of rats.

The group of animals whose average weight is represented by o received the basal diet only; those represented by ● received a little fresh milk per day as well. On the eighteenth day (represented by the dotted line) the diets of the two groups were interchanged. (From Hopkins.)

development when given daily a small quantity of fresh milk. These results are illustrated in the curves in Fig. 12, which are taken from the paper by Hopkins, referred to in the list of literature quoted at the end of the present chapter. Owing to its remarkable effect on growth, fat-soluble A is often called the growth vitamine.

But this effect on general growth is not its only function, for lack of it has been found to give rise to **rickets**—a disease in which the calcification of bones and teeth is markedly deficient. This has been shown by taking X-ray photographs of the skeletons of puppies fed on a diet from which the vitamine is absent. If a little fresh butter or

cod liver oil be added to the diet the disease can be prevented, and it can be cured by this addition if its ravages have not been too long continued.

The fat-soluble vitamine is present, as we have mentioned, in most natural oils and in butter, but it is absent from margarine. The reason for this is that the unsaturated liquid fats, which constitute the raw materials in the manufacture of butter substitutes, are heated in an atmosphere of hydrogen in the presence of finely divided nickel as a catalyst in order to produce saturated fats of higher melting point. The process is equivalent, for example, to the reduction of oleic acid to stearic acid (see p. 96). It only proceeds at a temperature which is high enough to bring about the decomposition of the vitamines originally present in the fat. A similar loss of vitamine occurs during the preparation of dry foods from milk.

Lastly, there is a third disease which is clearly associated with vitamine deficiency. This is the **beri-beri,** which is prevalent among eastern peoples such as the Japanese, whose staple article of diet is rice. The essential pathological change consists in the degeneration of the peripheral nerve trunks, so that loss of sensibility and muscular paralysis and degeneration occur. It has been found that this condition results only if the diet consists of white rice as we know it, with the reddish outer husk polished off. If the crude unpolished rice be eaten the symptoms of beri-beri do not arise. The condition can be readily imitated in birds by feeding fowls exclusively with polished rice: an extensive degeneration of the nerve tracts (neuritis) ultimately sets in, and after death the broken down nerve fibres can be readily detected histologically. It is possible to cure a bird, even one which is very seriously affected, by

giving it a little of the rice bran to eat or by giving it an injection of an extract of the polishings. This anti-neuritic vitamine is usually called **water-soluble B**, it is, as a matter of fact, present not only in the outer layers of the rice grain, but also in a large number of other tissues, both plant and animal. It is found to be ready soluble in alcohol, but its chemical constitution is unknown.

The facts mentioned in this section find their most important applications in the feeding of infants. So long as a child can receive its mother's milk it obtains vitamines enough; but when it is reared on artificial food the circumstance must be taken into account that the process of manufacture of such foods involves drying and heating and exposure to air—processes which lead to decomposition of the vitamines present. These substances must therefore be supplied to the infant in small quantities of fresh foods given to it in addition to a bulk of the milk product or other artificial food sufficient to supply the energy and material needs of its growing tissues. It is usual to supply the fat-soluble A, by giving small quantities of cod-liver oil, and the anti-neuritic and anti-scorbutic water-soluble vitamines by including a few c.c. of orange juice in the daily ration.

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Report on the Present State of Knowledge concerning Accessory Food Factors (*Vitamines*). (H.M. Stationery Office, London, 1919.)

Among the numerous original articles on the subject, the paper by Hopkins entitled

Feeding Experiments Illustrating the Importance of Accessory Factors in Normal Dietaries (*Journal of Physiology*, Vol. XLVI. (1912), p. 425),

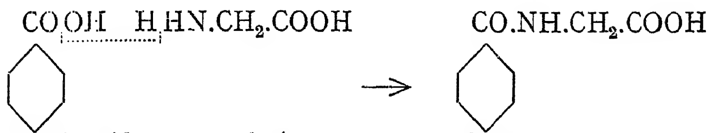
is particularly suitable for students' reading.

CHAPTER XV.

PROTECTIVE SYNTHESIS.

THERE are several ways in which the body can overcome the harmful effects of poisonous substances which are taken into it or are produced during the course of metabolism or bacterial action in the tissues. The formation of what are called anti-bodies, which combine with and neutralize the poisons or toxins liberated during the development of bacteria, is now-a-days a process so familiar as to form a piece of everyday knowledge. Another very important method which the body possesses for combating poisons is that known as protective synthesis. This is a process whereby a poisonous substance of comparatively simple chemical structure is built up in the body into a more complex product by which the poisonous properties of the original harmful substance are no longer exhibited so that the poison is masked until it can be eliminated from the body. To take an example—an animal is given a dose of benzoic acid. This is a poison which cannot be oxidised away in the tissues, so that it would tend to remain where it was carried and to exercise its poisonous action. But it is found that the benzoic acid does not long remain as such; it is very soon built up with glycine into a more complicated substance known as **hippuric acid,** which is a comparatively harmless substance, and is excreted by the

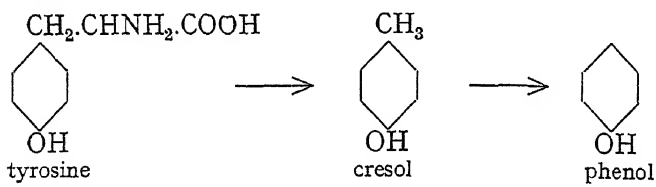
kidney. Hippuric acid is benzoyl glycine, its formation can be represented as follows:—

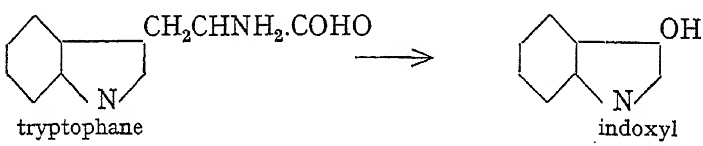


benzoic acid glycine benzoyl-glycine (hippuric acid)

That is a typical example of what we mean by protective synthesis. The body defends itself against the benzoic acid by synthesising from it a more complicated but less harmful product. It is not surprising that it should be glycine which is used in this way by the body, for there is always an abundance of glycine in the tissues; it is usually present in large amounts in the food, and even when it is not supplied the body can synthesise it (see page 79). Benzoyl-glycine is called hippuric acid because it occurs largely in the urine of the horse and other herbivorous animals. An animal that feeds mostly on vegetable food takes into its body with its diet quite a large amount of benzoic acid, and therefore uses this protective mechanism to a larger extent than an animal that eats little plant food. Thus we find more hippuric acid in the urine of a horse than in that of a man. This formation of hippuric acid furnishes the body with one method of protection against harmful ring compounds. But there are others. It is well known that in the alimentary canal bacteria abound. Now among the reactions brought about by intestinal bacteria the most important, from our immediate point of view, are the attacks on the molecules of tyrosine and tryptophane. The bacteria, as one may put it, "bite off" portions of the side chain of these amino-acids, and then the residual

substances still containing the ring intact are no longer capable of being oxidised in the body. We have already mentioned that tyrosine and tryptophane are exceptional among ring compounds in being completely oxidised by the body. A comparatively small change in the structure of the side-chain is sufficient to prevent the ring from being oxidised. The result of the bacterial action in the alimentary canal is the formation of such substances as **phenol** and **cresol** from tyrosine and **indoxyl** from tryptophane. The way in which these products arise will be clear from a study of the formulæ. Note that in each case the ring remains, but that the side chain has been attacked:—

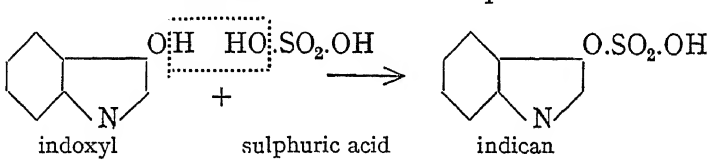




Now these phenols are too poisonous to be allowed to pass into the general circulation. But when these substances are absorbed from the alimentary canal, on reaching the liver they are coupled up with sulphuric acid, forming what are known as **ethereal sulphates.** The sulphuric acid radicle attaches itself to the hydroxyl group and so masks the poisonous properties this group possesses in these compounds. These ethereal sulphates of phenol, cresol and indoxyl are therefore much less poisonous than the original substances themselves; they

can be carried round the circulation without harm until they arrive at the kidney to be excreted in the urine. Evidently the amount of ethereal sulphates in the urine is a measure of the amount of these substances produced, and so will give an indication of the amount of bacterial decomposition occurring in the alimentary canal. Naturally this is increased during constipation. The output of ethereal sulphates is also notably increased as a result of the formation of poisonous products of the bacterial decomposition of proteins going forward in a large abscess.

One of these ethereal sulphates—indoxyl-sulphate or **indican**—is an interesting substance because it can be readily oxidised to indigo blue by means of hydrochloric acid and potassium chlorate solution. This is used as a test for indican in urine; the indigo produced can be made more evident by dissolving it out in chloroform by shaking the reaction mixture with a few drops of the solvent. The formation of indican is represented thus:—



The total amount of ethereal sulphate in urine is estimated by determining first of all the inorganic sulphate and then taking another sample of the urine, boiling with dilute hydrochloric acid to hydrolyse the ethereal sulphates, and then estimating the total sulphate, as before. This now includes both the sulphate of the inorganic sulphates and also that obtained from the ethereal sulphates. The inorganic sulphate having been already determined, the amount of sulphuric acid combined as ethereal sulphate is known.

There are two other ways in which the body may protect itself against poisons, but these are methods which are used more for combating foreign substance administered in the diet rather than for the elimination of poisonous products of metabolism. Such a substance as brombenzene—C_6H_5Br—is found to be got rid of in combination with the amino-acid cystine in a form of a substance known as **mercapturic acid.** And lastly, many of the drugs administered medicinally appear in the urine coupled with a substance called **glycuronic acid,** which is an oxidation product of glucose:—

$$\begin{array}{l} \text{COOH} \\ | \\ \text{CHOH} \\ | \\ \text{CHOH} \\ | \\ \text{CHOH} \\ | \\ \text{CHOH} \\ | \\ \text{CHO} \end{array}$$

glycuronic acid

This is the case with phenol itself which may find its way into the blood stream not only from the alimentary canal, as we have mentioned, but also from the excess of it present in a careless dressing on a wound. Such phenol is excreted in the urine, partly as ethereal sulphate, and partly as glycuronate, and, seeing that in the formation of the glycuronate the terminal oxidisable aldehyde group—CHO—of the molecule is not masked in any way, the presence of this substance in the urine confers upon the fluid the power of reducing copper solutions—for example, Fehling's—as if it contained sugar itself.

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The most accessible account of our present topic is
The Fate of Foreign Organic Compounds in the Animal Body, by C. P. SHERWIN. (*Physiological Reviews*, Vol. II. (1922), p. 238.)

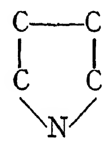
CHAPTER XVI.

THE PIGMENTS OF THE BODY.

"But what are their colours to me?"
A.D.C. Pantomime, Cambridge, 1921.

THE pigments of the body form a group of complex substances whose reactions will be of interest to anyone who has a taste for rather complicated chemistry. By far the most important of the pigments which occur in the animal body is **hæmoglobin**—the scarlet colouring matter of the red blood corpuscles. It owes its importance to three chief circumstances. In the first place, it plays so essential a part in the carriage of the respiratory gases, oxygen and carbon dioxide; secondly, it gives rise during its decomposition in the body to the pigments of bile and fæces, and to one of the pigments of the urine; and, thirdly and lastly, it gives rise to a number of derivatives and related products which are of interest not only from the strictly chemical point of view, but also because they occur abnormally in the body under the influence of poisons or disordered metabolic processes. We shall deal with each of these topics, but not in the order in which we have mentioned them. It will be more convenient to begin with a consideration of the chemical properties of hæmoglobin and its derivatives first, and then to consider the metabolic changes which these substances undergo in the body. We shall reserve the question of the carriage of the blood gases for the next chapter.

To begin, then, with a consideration of the chemistry of hæmoglobin itself. The first fact to be noticed is that this substance is a conjugated protein, that is, that its molecule consists of a compound of a protein, **globin,** with a non-protein moiety called **hæmatin.** Globin is a typical globulin, being insoluble in water, but soluble in salt solutions. Hæmatin is a complicated substance whose chemical constitution has not been settled completely, but which is known to contain one atom of iron and also four pyrrol rings in the molecule. We met the pyrrol ring in discussing the formula for tryptophane (p. 7); it will be remembered that it is a five-membered ring of which one member is a nitrogen atom:—



It is interesting to observe that this ring is also contained in chlorophyll, the green colouring matter which is essential for the photosynthetic process in plants. Hæmoglobin is exceptional among proteins in the ease with which it can be crystallised. In the case of the blood of some animals—notably that of the horse—it is necessary only to centrifuge off the red corpuscles, and then to disrupt them in some way, say, by treatment with a little ether, in order to liberate the hæmoglobin and so obtain a solution from which the pigment will crystallise out when it is kept in the cool. In other cases a little alcohol must be added in order that the hæmoglobin shall come out of solution.

The most characteristic property of hæmoglobin is its power of combining with oxygen to form the easily

dissociable compound **oxyhæmoglobin.** This is, in fact, the product obtained by the method of preparation just given, for the hæmoglobin, if not completely saturated with oxygen in the original blood, will become so on exposure to air during the manipulations. The oxygen may be removed from oxyhæmoglobin in order to form hæmoglobin itself—"reduced" hæmoglobin it is often called—by exposure to a vacuum or to an atmosphere devoid of oxygen, or by treating the oxygenated substance with reducing agents. Of these, yellow ammonium sulphide works very well if the solution be warmed slightly, or an alkaline solution of ferrous hydroxide called Stokes' fluid may be used in the cold. The reduced hæmoglobin is of a purplish colour, but this is not easy to see in solutions to which these reagents have been added. On shaking with air the hæmoglobin reabsorbs the oxygen and becomes converted into oxyhæmoglobin once more. As it is by means of their colours that these pigments are distinguished one from the other, it will be useful at this point to give an outline of the way in which this property is utilised for the purpose. That these substances possess colour at all depends upon the fact that when white light passes through their solutions, some of the coloured rays of which the white light is known to be composed, are absorbed, while the remaining coloured rays are relatively unaffected, and so, emerging from the solution, are visible to the eye. Or if the white light is first spread out by means of a prism into a spectrum so that its constituent rays of various colours, that is, of various wave-lengths, are all arranged in order, then, on viewing the spectrum through a layer of a particular pigment, this substance will absorb rays of certain particular wave-lengths so that only the remaining rays

will get through. We shall see, therefore, only certain parts of the coloured spectrum, the remaining portions where the corresponding rays have been absorbed by the pigment appearing as dark **absorption bands.** The part of the spectrum which is transmitted through the solution is known as its **absorption spectrum,** and, seeing that each pigment possesses its own characteristic absorption spectrum, the spectroscope furnishes the readiest means by which these substances can be identified. Actually in practice it is usual to allow the white light to pass first through the solution to be examined and then by means of the prism of a spectroscope to analyse the mixture of rays which passes through in order to determine which wave-lengths have been absorbed. If we observe carefully the spectrum formed by sunlight we find that it itself is not continuous, but is crossed by a number of fine black lines known after their discoverer as Fraunhofer lines. These are nothing more nor less than the very narrow absorption bands of substances which exist in the form of vapour in the atmosphere of the sun, and which absorb certain rays from the white light sent out from the glowing interior. One of the most conspicuous of these lines is that labelled D, which is known to be an absorption band of sodium vapour. This occurs at a certain wave-length whose value has been determined by physical methods to be 589 millionths of a millimetre (589 $\mu\mu$). The wave-lengths of the other easily visible Fraunhofer lines have also been determined, and by means of these it is possible to construct a scale of wave-lengths so that the position in the spectrum of any absorption band given by a pigment can be accurately defined. It is to be noted that this scale of wave-lengths will not possess divisions of equal size because a prism

spreads out the rays at the violet end of the spectrum to a greater extent than those at the red end.

To return now to the properties of the particular pigments we are studying—if we observe the absorption spectrum of oxyhæmoglobin—provided that the solution is not so strong as to block out the greater bulk of the spectrum altogether—it is easy to see that light is absorbed most strongly in two regions, fairly close together in the green. Oxyhæmoglobin has, therefore, a double-banded absorption spectrum.

On the other hand, reduced hæmoglobin shews only a single absorption band which overlaps the space included by the outer edges of the two bands of oxyhæmoglobin, as is seen in the spectra represented on the opposite page.

Now the most characteristic property of hæmoglobin is its power to combine with gases. Next in importance to its compound with oxygen is that which it forms with carbon monoxide. Indeed, hæmoglobin combines more readily with carbon monoxide to form **carboxy-hæmoglobin** that with oxygen to form oxyhæmoglobin, so that if oxyhæmoglobin be exposed to an atmosphere containing but a small proportion of carbon monoxide this gas displaces the oxygen from its combination and carboxy-hæmoglobin is formed. A sufficient percentage of carbon monoxide is contained in coal gas, so that if this be bubbled through diluted blood, carboxy-hæmoglobin is readily formed. This carboxy-compound differs in several respects from oxyhæmoglobin. Its solution is of a bluish-red colour and not a full scarlet. Its absorption spectrum shows two bands, in the green, but these are slightly nearer the violet end of the spectrum than those of oxyhæmoglobin, as may be seen from a careful comparison of the two spectra as given in the figure. A

simple test by means of which a solution of oxyhæmoglobin can be distinguished from one of the carboxy-compounds is to dilute each with water until the colour is faint. It is found that in extreme dilution the colour of the oxyhæmoglobin is yellow, while that of carboxy-hæmoglobin remains bluish-red until the dilution has been carried so far that colour can no longer be detected. Chemically, carboxy-hæmoglobin is distinguished from oxyhæmoglobin by the fact that it cannot be reduced to hæmoglobin by means of ammonium sulphide or Stokes' reagent.

Before leaving the immediate derivatives of hæmoglobin we must mention that when oxyhæmoglobin is treated with an oxidising agent—potassium ferricyanide is the one usually employed—the whole of its oxygen is liberated as gas. The reduced hæmoglobin so formed is however at once reoxidised by the ferricyanide, not with the re-formation of oxyhæmoglobin, but with the production of a brown substance isomeric with it, called **met-hæmoglobin.** This is an important reaction, because by collecting and measuring the amount of oxygen evolved the amount of hæmoglobin in any solution can be estimated. Met-hæmoglobin itself is interesting because it occurs in the blood of chemical workers who live in atmospheres containing nitrous fumes, or the vapour of nitro-benzene. Met-hæmoglobin can be reduced by ammonium sulphide or Stokes' reagent. Its most conspicuous absorption band is in the red, but there are also two faint ones in the green and a general absorption of the blue rays.

We have now dealt with the more important immediate derivatives of hæmoglobin in which the essential constitution of the molecule has remained undisturbed.

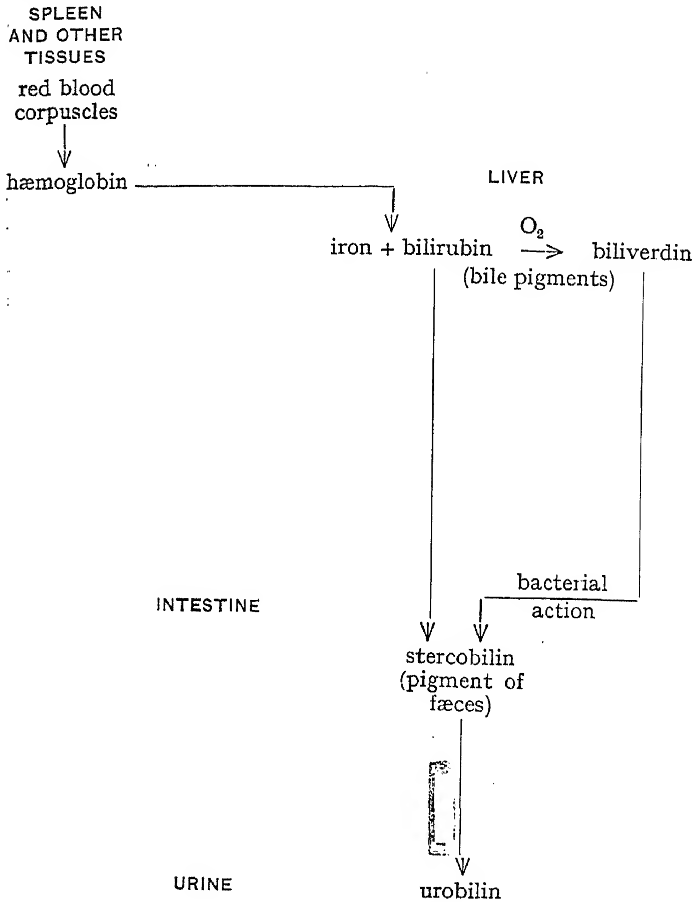
We now pass on to consider the substances obtained by breaking down the original complex molecule to varying degrees. In the first place we must notice that gentle decomposition by means of a dilute acid or alkali severs the union between the globin and the hæmatin so that these constitutents of the hæmoglobin molecule are liberated in the free condition. Globin, as we have already said, is a typical globulin, while hæmatin is a complex ring compound containing iron. Its solutions are brown in colour and show different absorption spectra according to whether they are acid or alkaline. **Acid hæmatin** can be extracted from its solutions by means of ether, while **alkaline hæmatin** is readily soluble in alcohol; in fact, one of the easiest ways in which to obtain a pure solution of alkaline hæmatin is by extracting a dried paste of blood and potassium carbonate with hot alcohol. Alkaline hæmatin can be reduced with ammonium sulphide, its reduction product being termed **hæmochromogen.** Hæmatin forms salts with acids; an acetyl derivative of its hydrochloric acid salt forms the brown **hæmin** crystals obtained by boiling a drop of blood with a fragment of sodium chloride and a little glacial acetic acid on a microscope slide—a reaction which is of importance as a test for blood.

Now hæmatin, hæmin and hæmochromogen all agree in retaining the iron of the original hæmoglobin in their molecules. But on treatment with strong acids this iron is removed and iron-free derivatives result. The most important substance so obtained is called **hæmatoporphyrin.** (Gr. πορφυρεος = purple.) It can be made direct from hæmoglobin by allowing a few drops of blood to fall into strong sulphuric acid, when a purple solution containing hæmatoporphyrin is obtained. The substance

itself can be precipitated by pouring the acid mixture into water.

The great interest of hæmatoporphyrin is that it is a derivative obtained by the removal of the iron atom from the hæmatin molecule. It has, therefore, important relationships with the pigments of the bile. For the hæmoglobin which is liberated by the breaking down of worn-out red corpuscles in the spleen and other tissues is carried ultimately to the liver. Here the pigment is decomposed, the iron is removed and stored in the liver cells, and the iron-free residues are converted into the bile pigments. The decomposition of the hæmoglobin in the liver follows almost the same course as it follows when it is acted upon by strong acids. But there is a certain difference between the two processes, for in the liver the iron-free substance formed is the reddish pigment **bilirubin,** which is isomeric but not identical with the product of the action of the acid, namely, hæmatoporphyrin. The green pigment of the bile, **biliverdin,** is an oxidation product of bilirubin, and differs from the latter merely in possessing two more oxygen atoms in the molecule. When these bile pigments pass into the alimentary canal they are attacked by the bacteria which abound in the intestinal contents. Apparently reduction then takes place, and nitrogen atoms are removed, the product being the brown pigment of the fæces. Of this pigment, however, a certain amount is absorbed into the blood stream, and is carried to the kidney, and so appears in the urine, where it is known as **urobilin.** This, it should be noted, is a pigment of only secondary importance in the urine, the chief yellow pigment being a substance called **urochrome,** whose chemical constitution is unknown. This substance

is derived, at least in part, from the chlorophyll contained in plant food. These relationships are expressed in the following scheme:—



In giving this brief account of the chemistry of these pigments it will be observed that we have refrained from giving chemical formulæ for the substances with which we have dealt for the reason that at present our knowledge

of the chemistry of these complex pigments is yet too meagre to enable us to do so with any degree of certainty. For example, it is not even settled whether the molecules of hæmatin, hæmatoporphyrin and bilirubin contain 32, 33 or 34 atoms of carbon, and it is evident that until so fundamental a matter is settled any formula put forward to describe the relationships of these substances will be nothing more than a suggestion or expression of possibilities which, while valuable and necessary in a larger treatise, would be out of place in a general survey such as it is our object to give here. The chief facts we have mentioned in this chapter may be summarised conveniently as follows:—

(reduced) **hæmoglobin**
(purple: a single absorption band in the green)

↑ ammonium-sulphide, Stokes' reagent | air ↓

met-hæoglobin (brown) ←ferricyanide— **oxyhæmoglobin** (scarlet: two absorption bands in the green) ——→ **carboxy-hæmoglobin** (bluish-red: two absorption bands in the green slightly nearer the blue than those of oxyhæmoglobin: not reduced by ammonium sulphide or Stokes' reagent)

↓ dilute acids and alkalis

hæmin ←hydrochloric and acetic acids— **hæmatin** + globin (brown) —ammonium sulphide→ **hæmochromogen** (reduced alkaline hæmatin: red)

↓ strong acids

hæmatoporphyrin + iron
(purple: isomeric with bilirubin: iron free)

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Our knowledge of the bio-chemistry of the blood pigments seems not to have been collected into a monograph. The most useful account of these substances is that given in Abderhalden's *Lehrbuch.*

Instructions for the preparation of hæmoglobin derivatives are given in Plimmer's *Practical Organic and Bio-chemistry*; for methods of obtaining them in small quantities for detection and spectroscopic investigation see Cole's *Practical Physiological Chemistry.*

CHAPTER XVII.

THE RESPIRATORY GASES.

"Nor is there any chemical substance which exactly resembles hæmoglobin....But for its existence man might never have attained any activity which the lobster does not possess, or had he done so, it would have been with a body as minute as the fly's."—*Barcroft.*

So far as the body is concerned, the most important property of hæmoglobin is its power of combining with oxygen in the lungs to form oxyhæmoglobin, and of liberating the oxygen again when this oxyhæmoglobin has been carried in the blood stream to active tissues where oxygen is needed. In this way the hæmoglobin subserves the essential function of transporting an abundant supply of oxygen to the internal organs which are cut off from direct contact with the external air. In this transport of oxygen between lungs and tissues we are dealing with a reversible chemical reaction. In the lungs it occurs in such a manner that oxyhæmoglobin is formed from its constituents, in a way which can be represented by an equation thus:

$$Hb + O_2 \longrightarrow HbO_2$$

if we allow the symbol Hb to stand for the whole complex molecule of hæmoglobin; while in the tissues the course of the reaction is reversed so that it is now to be represented as:—

$$HbO_2 \longrightarrow Hb + O_2.$$

Like all reversible chemical reactions, the direction in which this reaction will proceed is determined by the

concentrations of the substances taking part. In the body the chief factor which determines whether oxyhæmoglobin shall be formed or shall be dissociated is the tension or concentration of oxygen. In the lungs the blood is exposed to a relatively high tension of oxygen and oxyhæmoglobin is formed—the oxygen is present in such concentration that it can force itself, so to speak, on the hæmoglobin. On the other hand, in the active tissues the oxygen is being used so rapidly that there is very little accumulated concentration of it, the oxygen molecules are present in such small concentration that they are powerless to resist the tendency of the hæmoglobin to throw them off, and therefore the oxyhæmoglobin now dissociates.

It is evident that in order to study the efficiency with which this transport of oxygen is carried out, that is, the amount of oxygen which each cubic centimetre of blood can carry between the lungs and the tissues—we must know not only the tensions of oxygen in lungs and tissues, respectively, but also the amount of oxygen which is taken up by the blood at each particular value of oxygen tension. We must expose blood to various tensions of oxygen, and when equilibrium has been obtained we must determine by means of analysis the amount of oxyhæmoglobin which has been formed in the blood at each particular tension of oxygen. On plotting these results we obtain a curve known as the dissociation curve of oxygen in blood, or more briefly the **oxygen dissociation curve of blood,** from which we can read off at a glance the amount of oxygen in a standard volume of blood at any particular value of oxygen tension and can therefore easily determine the amount of oxygen which will be given off as the blood passes from a higher to a

lower oxygen tension. Now ordinary atmospheric air contains about one-fifth of its volume of oxygen. Its tension, or partial pressure of oxygen will, therefore, be about $\frac{1}{5}$ of an atmosphere or $\frac{1}{5}$ of 760 millimetres of mercury, i.e. about 152 mm. Hg. Owing to the continuous absorption of oxygen by the blood, the air in the lungs will contain a smaller percentage, and therefore a smaller tension of oxygen than this inspired air —not much above 100 mm. of mercury. It is evident that since this is the highest oxygen tension that the blood meets in the body this will be the highest tension of the gas to which we need plot the oxygen dissociation curve if we are dealing with a normal body breathing normal atmospheric air. All the other values of oxygen tension which we shall need to use will be smaller than this, and will be obtained most conveniently by diluting ordinary atmospheric air to suitable extents with nitrogen in order to reduce the percentage and therefore the tension of oxygen in the mixtures. Suppose we have prepared a series of such mixtures possessing oxygen tensions ranging from small values up to about 100 mm. Hg. We then expose a small quantity of blood to each mixture by shaking in a suitable vessel—immersed in water at 37° C. if we wish to work at body temperature—and when equilibrium has been obtained we withdraw a measured sample of the blood and determine how much oxygen it contains in the form of oxyhæmoglobin by liberating and measuring the gas either by means of a vacuum pump or by treating with potassium ferricyanide (see p. 199). On plotting the results obtained in a series of such measurements—marking off values of oxygen tension along the horizontal direction and the total amount of oxygen present in 100 c.c. of the blood

vertically—we obtain the curve shown in the following figure:—

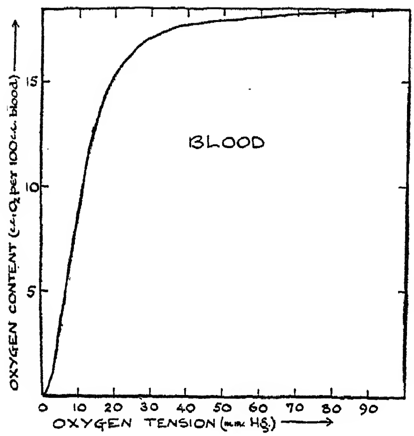

FIG. 14. The oxygen dissociation curve of normal human blood at body temperature and in the absence of carbon dioxide.

The curve is S-shaped. (From Barcroft's results.)

It is seen that the oxygen dissociation curve has a complicated S-shape, the amount of oxygen taken up by 100 c.c. of blood increasing at first slowly and then more rapidly as the oxygen tension is increased, and lastly, tending slowly to reach a maximum of about 18·5 c.c. of gas per 100 c.c. of blood at the highest values of oxygen tension investigated. Of course, of this oxygen a small amount is present in physical solution, as it would be in water, but this dissolved quantity only amounts to about a third of a c.c. at 100 mm. Hg. oxygen tension. So that, practically speaking, the whole of the oxygen carried by blood is in the form of oxyhæmoglobin.

This S-shaped curve is evidently the expression of an equilibrium much more complicated than is described by the simple reversible equation already mentioned—

$$Hb + O_2 \rightleftarrows HbO_2.$$

In order to study the influence of the various factors at

work in the blood determining this complicated relationship between oxygen and hæmoglobin let us perform an exactly similar experiment with a solution of hæmoglobin in pure water in place of blood, and plot out the oxygen dissociation curve for this. We then obtain a very different curve, as the following diagram of it shows:—

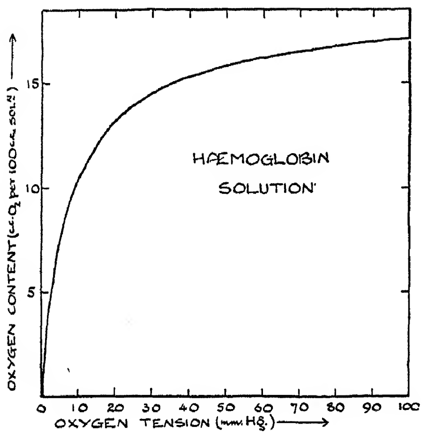

FIG. 15. The oxygen dissociation curve of a solution of hæmoglobin at body temperature.

The curve is a hyperbola. (From Barcroft's data.)

This curve is much simpler than that given by the blood. The degree of combination with oxygen of the hæmoglobin in aqueous solution increases at first rapidly as the oxygen tension is increased, and then changes gradually more and more slowly until the same maximum is reached as with the same concentration of hæmoglobin in blood. This curve which expresses the equilibrium between oxygen and hæmoglobin in pure aqueous solution, is found to be a rectangular hyperbola—which is what a chemist would expect if the reaction followed the simple equation and the oxygen and hæmoglobin combined simply molecule for molecule. It is necessary to find some explanation of the more complicated result

obtained when the hæmoglobin is present, not in aqueous solution, but in the corpuscles of blood. It has been discovered that the reason for the difference is that in the corpuscle contents the hæmoglobin is in a solution containing various salts, and that these have a marked effect on its combining power for oxygen. They do not influence very much the maximum amount of oxygen with which the hæmoglobin will combine at high oxygen tensions, but they so alter its properties that it will combine with appreciably less oxygen at low oxygen tensions. In other words, the salts tend to cause the dissociation of oxyhæmoglobin at low oxygen tensions, and so to assist in the discharge of oxygen from the blood to the tissues. The dependence of the combination of hæmoglobin with oxygen upon the character of the salts present has been shown by some very ingenious experiments. It has been found that while the blood of every mammal investigated gives an S-shaped oxygen dissociation curve, the exact proportions of the curve vary from one animal to another. For example, the oxygen dissociation curve of dogs' blood has a somewhat different shape from that of human blood when the two curves are plotted on the same scale. That the differences between these curves are due to differences in the salts present in the corpuscles of the two species is shown by the observation that if a solution of hæmoglobin in distilled water is divided into two portions, and to one portion the salts known to be present in human red corpuscles are added in appropriate amount, while in the other, the salts characteristic of dogs' corpuscles are similarly dissolved, then it is found that the first mixture gives the same oxygen dissociation curve as normal human blood, while the second portion has properties identical

with those of dogs' blood, so far as combination with oxygen is concerned.

Further, the properties of hæmoglobin are influenced not only by salts, but also by other electrolytes, such as acids and alkalis. Acids produce a very marked increase in the ease with which oxyhæmoglobin dissociates at low oxygen tensions, without affecting very much the total amount of oxygen with which the hæmoglobin will combine at high tensions. As one of the chief products of the oxidation in tissues is itself an acid—namely, carbonic acid—we can see in this behaviour a further mechanism which will assist in the unloading of the oxygen from the blood as it passes through active tissues. For the more active the tissue the more carbon dioxide it produces, and therefore the more the dissociation of the oxyhæmoglobin brought to it will be accelerated. Owing to this effect, in order to imitate the conditions found in the body as closely as possible, we ought to determine the oxygen dissociation curve of blood in the presence of that tension of carbon dioxide which is known to exist in the blood in the body. In arterial blood there is usually a tension of approximately 40 mm. Hg. of carbon dioxide. The oxygen dissociation curve for blood exposed to a constant tension of carbon dioxide of this amount is given in Fig. 16. In it also we have copied the curve previously given in Fig. 14 for blood containing no carbon dioxide. It is easy to see how the carbon dioxide diminishes the amount of oxygen the hæmoglobin will hold at low oxygen tensions while leaving almost unaffected the amount it can combine with, at tensions of oxygen such as exist in the lungs. Also in Fig. 16 we give the oxygen dissociation curve given by a solution of hæmoglobin in pure water

not at body temperature but at average room temperature (16° C.). By comparing this last-mentioned curve with that given in Fig. 15, which was determined at body temperature, it will be seen that the effect

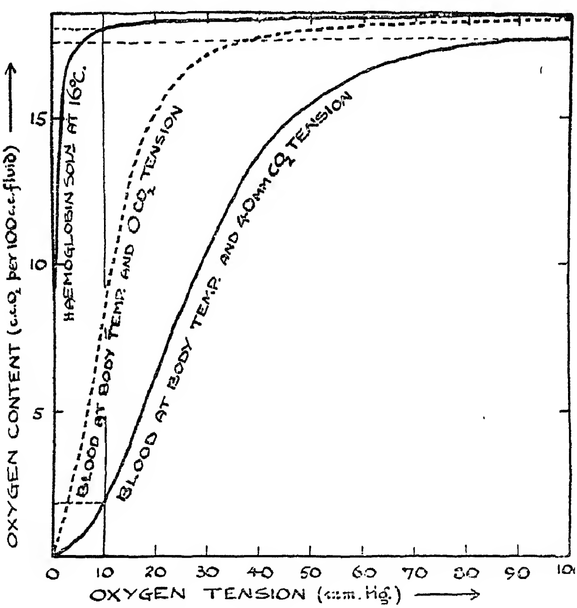

Fig. 16. The oxygen dissociation curve of human blood at body temperature, and at the normal arterial tension of carbon dioxide (40 mm. Hg.), compared with the curve for blood free from carbon dioxide, and with the curve for a solution of hæmoglobin at room temperature (16° C.).

The superior oxygen carrying power of the blood at body temperature is shown by the extent to which it dissociates its oxygen at the lower tensions of the gas. (From Barcroft's data.)

of increase of temperature also is to assist in the dissociation of oxyhæmoglobin, so that we may say that the relatively high temperature of the body, the presence of salts and of carbonic acid all help in the

discharge of oxygen from the blood as it courses through the capillaries of an active organ. What a great advantage with respect to the carriage of oxygen the whole warm blood possesses over a plain cold solution of hæmoglobin may be seen by comparing in Fig. 16 the amount of oxygen which would be given off if 100 c.c. of the hæmoglobin solution and of the blood were each brought from an oxygen tension of (say) 100 mm. Hg. to one of 10 mm. It will then be seen that while the hæmoglobin solution has given up only ·6 c.c. of oxygen the blood will have given up no less than 15·7 c.c. We could wish for no better demonstration of the way in which the properties of hæmoglobin are modified by the conditions obtaining in the body so that the transport of oxygen from lungs to active tissues shall be carried on with the efficiency which is necessary for the maintenance of the vigorous life which a warm-blooded creature lives.

Before we leave the question of the carriage of oxygen by the blood we should mention that, in plotting the oxygen dissociation curves we have given, we have expressed the total quantity of oxygen present in the blood under any given conditions in c.c. of gas per 100 c.c. of blood. This method has the merit of clearness, but it is usual to find oxygen dissociation curves in which the quantity of oxygen present is expressed as a percentage of the total amount of oxygen with which the blood will combine at a high oxygen tension. This is called the **percentage saturation** of the blood with oxygen. This method of expressing the total oxygen content of blood is adopted because it is somewhat easier to measure the relative degree of saturation of the hæmoglobin with oxygen than the total actual volume of oxygen

present. But this nomenclature should cause no difficulty when it is remembered that in normal blood a percentage saturation of 100 corresponds to a total actual content of oxygen of 18·5 c.c. per 100 c.c. of blood, and so on in proportion.

The study of the carriage of carbon dioxide by blood follows the same lines as the investigation of the carriage of oxygen. We can plot out **carbon dioxide dissociation curves** for blood by methods analogous to those used to obtain the oxygen dissociation curves. We expose blood to increasing tensions of carbon dioxide and then estimate the total amount of carbon dioxide present per 100 c.c. of blood by withdrawing a measured sample and extracting the carbon dioxide by means of the vacuum pump or by expelling it from the blood by adding acid. Seeing that carbon dioxide exerts so powerful an influence on the oxygen dissociation curve of blood it is not surprising that oxygen should produce a marked effect on the course of the carbon dioxide dissociation curve. Indeed, it is found that the actual carbon dioxide dissociation curve given by a sample of blood depends on whether or not the blood contains oxygen. If it be free from oxygen it will take up appreciably more of carbon dioxide at a given tension of the gas than if it be completely oxygenated and exposed to the same carbon dioxide tension. We thus obtain two carbon dioxide dissociation curves, one for reduced and one for fully oxygenated blood, of which the latter runs at a certain distance below the curve for the reduced blood throughout its course. These two curves are shown in Fig. 17. In this effect of oxygen on the carrying power of blood for carbon dioxide we see an adaptation which enables the carbonic acid to be carried from the tissues and to be

discharged from the lungs at a greater rate than would otherwise be possible. For as the blood becomes reduced in the active tissues it becomes the more capable of combining with carbon dioxide. On the other hand the oxygen taken up by the blood in the lungs tends to expel the carbon dioxide—oxygenated blood being capable of combining with less carbon dioxide than reduced blood

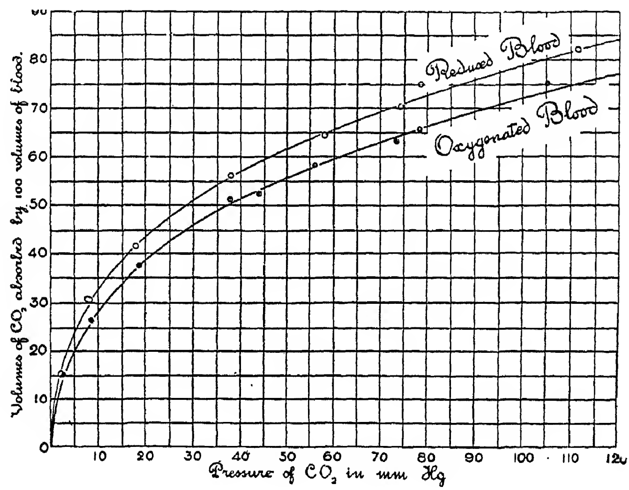

FIG. 17. The carbon-dioxide dissociation curves for oxygenated and reduced blood.

Note the extra carbon-dioxide taken up by the reduced blood at all tensions of the gas. (From Christiansen, Douglas and Haldane.)

at the same carbon dioxide tension. We have already mentioned that the carbon dioxide tension in the arterial blood is about 40 mm. Hg., and this is the lowest tension of carbon dioxide which the blood ever possesses in the normal body. From the dissociation curves just given it is seen that at this carbon dioxide tension 100 c.c. of the fully oxygenated blood contain over 50 c.c. of carbon

dioxide, so that even arterial blood, which contains less carbon dioxide than any other blood in the body will give off half its volume of this gas on acidification. Of this gas a certain small fraction will be in physical solution, but even though carbon dioxide is a fairly soluble gas at the tensions at which we are working this dissolved portion will not amount to a very appreciable fraction of the whole of the gas taken up. It is evident, therefore, that the gas combines chemically with some constituent of the blood just as does oxygen. There has been considerable discussion as to the exact mode of combination of carbon dioxide in blood, but there is good evidence for believing that the whole of the combined portion is in the form of sodium bicarbonate. We shall discuss this evidence more fully on a later page; at present we shall state simply that the sodium required for the formation of this sodium bicarbonate is supposed to come mainly from the molecule of hæmoglobin, which is a weak acid, and exists as its sodium salt in the weakly alkaline blood. When this is acted upon by carbonic acid, the sodium is removed from the hæmoglobin and goes to form sodium bicarbonate; in the lungs the carbonic acid escapes, and the sodium salt of hæmoglobin is once more formed. Sodium bicarbonate is a readily diffusible substance, and so when it has been formed from the hæmoglobin of the blood corpuscles some of it diffuses out into the plasma so that the combined carbon dioxide of the blood is not confined to the corpuscles as is the combined oxygen. The reason why oxygenated blood takes up somewhat less carbon dioxide than does reduced blood at the same carbon dioxide tension is said to be that oxyhæmoglobin is a somewhat more powerful acid than reduced hæmoglobin, and so holds more firmly to

the sodium, and gives off somewhat less of it to form bicarbonate with the carbonic acid.

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CHAPTER XVIII.

SOME APPLICATIONS OF PHYSICAL CHEMISTRY: GAS TENSIONS: OSMOTIC PRESSURE.

"I am convinced that biological chemistry cannot develop into a real science without the aid of the exact methods offered by physical chemistry."—*Arrhenius.*

Gas Tensions.

In our discussion of the carriage of oxygen and carbon dioxide by blood we frequently referred to the tension exerted by a particular constituent of a gas mixture, or to the tension of a gas in a liquid, but we did not turn aside from our main argument in order to explain and define these terms. The tension or partial pressure of a gas is that property which determines whether it shall diffuse into or escape from a space to which it has access. If the tension of a gas at any place is high, then the gas will tend to pass by diffusion to places where the tension is lower; conversely, a region of low tension will receive the gas from a situation where a higher tension prevails. The tension of a gas is therefore a factor analogous to temperature; for it is the relative temperatures of bodies which determine the direction of flow of heat between them.

Now, according to modern kinetic theory, this tendency to diffusion shown by a gas is an expression of the ceaseless energy of movement of its molecules, and the tension it exerts is the sum total of all the small elements of pressure

exerted by the separate molecules as, in their movement, they happen to collide with the walls of the space in which the gas is contained. If we have a mixture of gases in a given space, then the tension of each constituent is determined by the total rate of bombardment of the confining walls by molecules of that particular constituent. Now it is evident that the rate of bombardment by a molecule of any particular kind depends upon the number of such molecules present in each cubic centimetre of the gas mixture, and that this number may be varied in two ways—either by varying the composition of the gas mixture while keeping its total pressure constant or by changing the total pressure, and so, for example, compressing into a smaller space a greater number of molecules of the particular constituent in which we are interested, and, incidentally, of the other constituents as well. It therefore follows that the tension exerted by any one of a mixture of gases is determined both by the relative amount of that particular gas present in the mixture, and also by the total pressure exerted by the mixture. In symbols we may say that if the percentage by volume of the given constituent of the mixture be x, and the total pressure exerted by the mixture be p, then the tension of the particular constituent will be x per cent. of p. And this tension will be exerted unchanged, no matter how many molecules of other gases may be present in the mixture as well. For the spaces between the molecules of gases are so large that the molecules of one gas may move between the molecules of a second constituent without mutual interference—at least at moderate pressures.

In the case where one constituent of a gas mixture is the vapour of a liquid the same considerations hold; the

tension exerted by the vapour is determined by the relative proportion of it present. But in the case where there is also some of the liquid itself present, when, as it is said, the vapour is saturated, then the tension exerted by the vapour is no longer variable; it is a fixed quantity, which is determined solely by the temperature. If we attempt to increase the tension of vapour, say by compression of the mixture, the excess of vapour condenses, and the amount of liquid is increased; on the other hand, if the tension of the vapour is below the saturation value, then some of the liquid already present evaporates until the saturation value is reached. The tension exerted by the vapour therefore remains constant as long as there is some of the liquid present. These considerations are important in dealing with gases which are saturated with water vapour, such as air expired from the lungs, or air which has been exposed to blood and other aqueous fluids during experiments. The amount of the saturated water vapour pressure corresponding to the prevailing temperature must be subtracted from the total pressure exerted by the moist mixture, and only the remaining pressure is to be shared between the other constituents, according to their relative percentages by volume, in order to determine their respective tensions.

To take a numerical example—suppose that of the total volume occupied by the oxygen, nitrogen and carbon dioxide in a sample of alveolar air, 6 per cent. is occupied by the carbon dioxide. Suppose also that the barometer stands at 760 mm. Hg. In the lungs this air was saturated with aqueous vapour at body temperature (37°C.), since it was in contact with the moist lung surface. It is known that at this temperature the tension exerted by saturated aqueous vapour is 47 mm. Hg.

Therefore the remaining tension to be distributed between the oxygen, nitrogen and carbon dioxide is 760–47 =713 mm. Hg. As the carbon dioxide forms 6 per cent. of this mixture of the three gases, the tension it exerts will be, therefore, 6 per cent. of 713 mm. Hg., which is about 42·6 mm. Hg.

In defining the tension of a gas dissolved in a liquid the same considerations apply as in the case where it forms a constituent of a gas mixture. The tension of the gas is made up of the pressure exerted by its molecules as they strike the liquid surface. In order to arrive at a quantitative expression let us consider what happens when a liquid containing a gas in solution is brought into contact with a space also containing the gas. If the tensions of the gas in the liquid and in the gas space are such that in a given time more gas molecules strike its surface from below than from the gas space above, there will be a tendency for more gas molecules to pass through the surface from liquid to gas than pass into the liquid from the gas. The liquid therefore loses some of the gas to the gas space. On the other hand, if the tensions in the two phases are such that more gas molecules strike the surface from above than from the liquid below, then more molecules will pass from the gas space into the liquid than from liquid to gas; in this case the liquid gains gas from the gas space. But for a certain concentration of gas in the liquid, the number of molecules passing in a given time through the surface in one direction is just equal to the number passing in the same time in the opposite direction, so that no apparent change in the distribution of the gas between the two phases takes place. When this condition of equilibrium is fulfilled the tension of the gas in the liquid is said to be equal to that in the

gas space. The tension of a gas in a liquid is therefore defined as being equal to the tension of the gas in the gas space with which the liquid is in equilibrium. Measurements of the tensions of gases in biological fluids are important in physiological studies; for example, an accurate knowledge of the tensions of the gases in arterial blood is of prime importance in deciding the nature of the activity of the lung membrane. For if it should happen that under any conditions the tension of oxygen in the arterial blood leaving the lungs becomes greater than that in the alveolar air on the other side of the lung membrane, it is certain that any oxygen which then passes into the blood from the alveolar air cannot pass by a process of physical diffusion; it must be forced up the slope of oxygen tension by some active process for which energy is supplied by the cells forming the lung membrane itself. We do not wish to discuss this topic more fully here; we will content ourselves with a reference to the principle of the methods by which these measurements are made. It follows at once from our previous considerations. The liquid is shaken up or otherwise efficiently exposed to a small bubble usually of air until, by diffusion, the gases in the bubble have come into equilibrium with those in the liquid. Then all that is necessary is to analyse the bubble and to calculate from the results of the analysis the tensions of the various gases in it; the tensions of these gases in the blood or other liquid are equal to those in the equilibrated bubble.

Osmotic Pressure.

We have many reasons nowadays for believing that there exists some kind of attractive force between the molecules of a dissolved substance and those of the

solvent in which it is dissolved—an attractive force which has to be overcome whenever we separate some of the pure solvent from the solution. For this involves the separation of solvent molecules from the molecules of dissolved substance which are left behind in the more concentrated residue of the solution. Suppose, for example, that we have some salt dissolved in water, and we consider the separation of the water by spontaneous evaporation. We find that the vapour pressure of the solution at any temperature is appreciably less than that of pure water at the same temperature, the difference being a measure of the energy with which the molecules of salt attract the molecules of water. Again, suppose we wish to separate the solvent by boiling, then we find that in consequence of the diminished vapour pressure of the solution we have to heat it to a higher temperature than the boiling point of pure water in order that its vapour pressure shall be equal to that of the atmosphere, and that the liquid shall, in consequence, boil. This **elevation of the boiling point** is, then, another measure of the amount of work required to separate the water from its dissolved solid. Lastly, if we cool the solution, pure ice at first separates out. This constitutes yet another method of separating pure solvent from the solution, and again we find that there is greater difficulty in obtaining the ice from the solution than from the pure solvent for the solution requires to be cooled to a temperature appreciably below 0° C. before it begins to freeze. We have therefore a **depression of the freezing point** of the solution below that of the pure solvent, a depression which is a measure of the work required to be done in separating the salt from the water molecules.

Lastly, we can, theoretically, at least, separate the water from the salt solution by means of pressure, provided we can find some membrane which will act as a kind of sieve and will allow molecules of water, but not molecules of salt, to pass through it. Then we can apply pressure to the solution on one side of the membrane, and by this means cause water to pass through, leaving a stronger solution behind. One can imagine some such arrangement as is shown in Fig. 18, in which the special **semi-permeable membrane** forms the bottom of a cylinder in which the solution can be submitted to pressure by means of a piston. In this process of pressure filtration, just as in the process of evaporation already mentioned, we have to perform work in separating the pure solvent from the solution; in this case this is shown by the fact that we have to exert extra pressure on the piston over and above that which would be required to force water through the membrane if the cylinder were filled, at the beginning, with pure water, instead of a salt solution. The extra pressure which has to be exerted is termed the osmotic pressure of the salt in the solution. This osmotic pressure is an appropriate subject of study for the physiologist, because we find that most processes of secretion involve the separation from the blood, through a membrane or a cell, a solution more or less dilute with regard to at least some constituents than the blood which is supplied on the other side of the membrane.

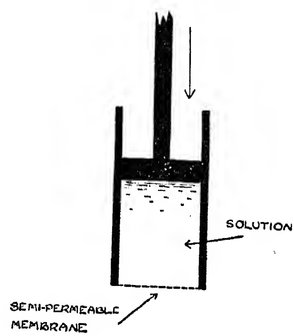

FIG. 18.

The existence of this osmotic pressure makes itself evident in another way. Suppose we have our solution in a cylinder, the end of which is closed by a semi-permeable membrane, as before. Let us, however, discard

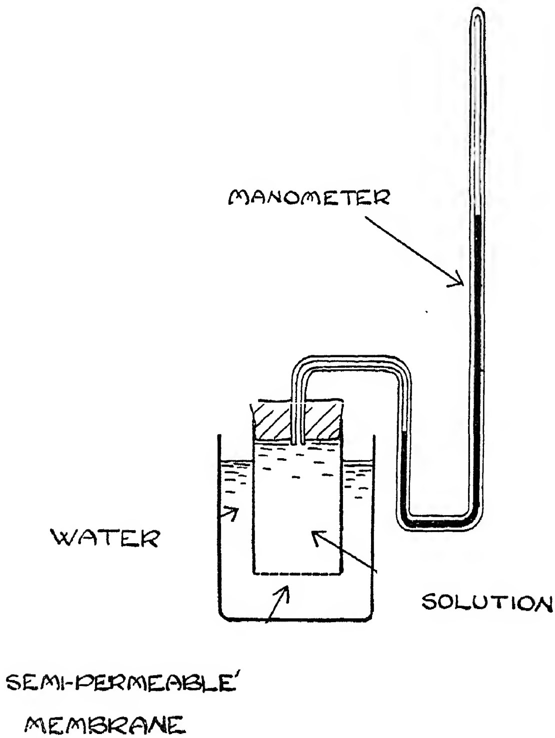

FIG. 19. Apparatus for direct measurement of osmotic pressure.

the piston, and instead close the upper end of the cylinder with a cover which carries a manometer of some kind. The cylinder being filled with a salt solution, let the whole then be immersed in a vessel of pure water as in Fig. 19. Under these circumstances diffusion of water molecules

will take place through the membrane, which is supposed to be freely permeable to them. Some molecules will diffuse into the cylinder from outside; others will tend to pass out into the surrounding mass of fluid. If we had pure water both inside and outside the cylinder we should have as many water molecules diffusing in as diffusing out, and the whole system would remain in equilibrium, no pressure change being recorded by the manometer. But in the case where the cylinder contains a salt solution, the water molecules inside will be at a disadvantage, for they are attracted back into the cylinder by the salt molecules which themselves cannot get through the membrane. Thus it comes about that more water molecules will diffuse into the cylinder in a given time than will diffuse out. There will, therefore, be an accumulation of water inside the cylinder which will give rise to an increase of pressure. But this increase of pressure will tend to squeeze out mechanically more water molecules through the membrane. So that the increase of pressure in the cylinder will continue until the assistance given by the pressure to the inside water molecules is just sufficient to compensate for what they lose owing to the back attraction of the salt molecules. Equilibrium will then be obtained, the inside water molecules, assisted by the pressure, escaping from the cylinder in equal numbers to those which enter. The constant pressure now registered by the manometer is thus equal to the pressure required to separate the inside water molecules from their accompanying salt molecules; it is, then, the osmotic pressure of the salt in solution. This is the principle of the method by which osmotic pressure is directly measured. In actual practice the cylinder we have described is furnished by an ordinary unglazed

porous pot; it is found that this can be rendered semi-permeable—at least, in the case of some solutions—by precipitating in its pores a layer of copper ferrocyanide, by filling it with copper sulphate solution and immersing it in a solution of potassium ferrocyanide. The two solutions meet by diffusion in the middle of the wall of the pot, and these react with the formation of a layer of the copper ferrocyanide precipitate which converts the pot into a semi-permeable membrane. After thorough washing it is necessary merely to fit a stopper and a suitable manometer and the apparatus is ready to give direct readings of the osmotic pressure exerted by any dissolved substance which will not pass through the membrane.

Many living cells seem to be bounded by membranes which, if not completely semi-permeable, are very limited in their permeability to many dissolved solids. Red blood corpuscles furnish us with an example. When these are placed in a salt solution which is more dilute than their contents, water passes into them—osmotically—and they swell and ultimately burst. The hæmoglobin is thereby liberated into the surrounding liquid, with the result that the mixture possesses a transparent red colour, and comes to differ very markedly in appearance from the original opaque blood. When the red corpuscles have been broken up in this fashion the blood is said to have been laked. It should be mentioned that this osmotic process is not the only method whereby blood corpuscles can be laked. The corpuscle membranes can be ruptured not only by bursting owing to treatment with a hypotonic solution, but also by many reagents which will attack them chemically. Ether and saponine may be mentioned among these. The venom of some snakes

produces extensive hæmolysis in a similar fashion. Then again, the sharp points and edges of the ice crystals formed when blood is frozen pierce the outer membranes of the red corpuscles and liberate their contents, so that alternate freezing and thawing finally results in the complete laking of the blood. On the other hand, if the blood corpuscles are placed in a stronger solution, water is attracted out through their membrane, with the result that they shrink. There is, however, one particular molecular concentration of salt which will not cause a change in the water content of the corpuscles, so that their volume remains unchanged. Such a solution is said to be **isotonic** or **isosmotic** with the corpuscles. If made up with sodium chloride it is usually referred to as normal saline; it will contain about ·6 per cent. of the salt if it is isotonic with frogs' corpuscles, and a slightly larger concentration —·7 per cent.—if it is to be normal for those of a mammal. In this they will remain in a normal condition. And what we have said with regard to blood corpuscles is true of cells in general. From this it will be evident that one of the chief requirements of a fluid which shall maintain in as nearly normal a condition as possible the structure and functional activity of a tissue, will be that it shall possess a suitable osmotic pressure.

The membranes we find in the body differ in several respects from inorganic precipitation membranes, such as the one we have just described. In the first place they are not often so completely impermeable to dissolved crystalloids as is copper ferrocyanide, for many secretions contain salts in somewhat the same concentrations as in blood. And again, an animal membrane is usually a cellular structure whose semi-permeability depends not on mere physical properties, but on active

processes occurring within the cells of which it is composed. That is to say, the cells forming the membrane in question constitute not only the semi-permeable structure itself, but also they supply from their own oxidative processes the energy which is required to bring about the filtration,—the cell representing, so to speak, both cylinder and piston in the mechanical model we described in the opening sentences of this section.

Of course, in any case where a particular constituent of a solution is not kept back by a membrane, but comes through in unaltered concentration, it is evident that since no concentration of this substance has taken place there has been no separation of solvent from this particular solute, and therefore no energy has had to be supplied in order to overcome that part of the osmotic pressure due to this constituent. We mention this because this is one of the conditions which we believe to obtain in the glomeruli of the kidney. The membrane separating the blood in the glomerular vessels from the cavity of Bowman's capsule is supposed to be impermeable to the blood proteins, but to be capable of allowing the unrestricted passage of salts, sugar and urea. If the glomerulus, then, is to act merely as a passive filter, the driving force of which is the blood pressure, it follows that it is the osmotic pressure of the blood proteins only which is the opposing force to the filtration. More exactly we should say that it is the osmotic pressure of the plasma proteins, since the hæmoglobin is confined to the corpuscles, and so is not free to be filtered off in any case.

Now the osmotic pressure exerted by the plasma proteins has been measured directly. For this purpose it was not necessary to use a very special membrane, as most animal membranes are impermeable to protein, although, in the

dead condition at all events, they are freely permeable to salts. Parchment serves excellently for the purpose. If an osmometer be constructed of this material and filled with blood plasma it is found that at first on immersing it in water a fairly high osmotic pressure is recorded. But the value of this pressure gradually falls as the salts originally present in the plasma diffuse through the membrane to the outside. After a time, however, when this diffusion has come to a standstill, the osmotic pressure will remain steady, its value being that due to the indiffusible proteins of the plasma, the salts and other diffusible substances being now in equal concentrations on both sides of the membrane, and so producing no effect. The value of the osmotic pressure so obtained is very small compared with that exerted by salt solutions of ordinary strengths. It amounts to about 30 mm. of mercury—three centimetres only.

This, then, is an estimate of the pressure which will oppose filtration from the blood plasma of a solution containing its crystalloid substances in unaltered concentrations, but from which the plasma proteins have been separated by the filtration membrane. On the other hand, the pressure available for the filtration is the difference of pressure of the blood in the glomerular capillaries and that of the urine in the cavity of the capsule. The pressure in the capillaries will be but little below the general blood pressure, particularly as these are relatively wide vessels which narrow considerably only as they leave the glomerulus. The pressure of fluid in the capsule will be but small, for the contractions of the ureter remove the urine as fast as it is formed. We conclude, therefore, that under normal circumstances the available filtration pressure will be able easily to

overcome the opposing osmotic pressure of the plasma proteins so that the glomerulus and capsule together constitute a pressure filtration apparatus. It has been found that when the effective filtration pressure is reduced —as it may be by a fall of blood pressure or by an increase of pressure on the ureter side of the glomerular membrane —then the formation of urine comes to a standstill when the difference between the blood pressure and ureter pressure is about 40 mm. Hg., that is to say, when the effective filtration pressure is just about balanced by the osmotic pressure of the plasma proteins. From this it is concluded that the main process in the formation of urine is one of filtration, for which the necessary energy is supplied by the heart in maintaining the blood pressure. It is also evident that with a given blood pressure the filtration will be the easier the lower the osmotic pressure of the plasma proteins. Now the osmotic pressure exerted by any substance in solution is, in general, very nearly, and in the case of dilute solutions, practically exactly, proportional to its concentration. If then we inject a considerable quantity of saline solution into the blood, the blood pressure may be kept constant by the regulatory activity of the vasomotor centre, but nevertheless a very increased formation of urine takes place owing to the fact that the saline has diluted the plasma, and so has reduced the osmotic pressure which its proteins oppose to the filtration. It is interesting to find also that this saline diuresis is not accompanied by any increase in the amount of oxygen used per minute by the kidney, which shows that the energy used in the extra filtration is not derived from oxidations in active kidney cells, but is obtained from a source external to the kidney, i.e. the heart-beat. The student will now easily realise why,

in order to compensate for the great fall of blood pressure occurring during conditions of shock, it is useless to inject a plain saline solution. This would dilute the plasma and so reduce the osmotic pressure which its proteins oppose to the filtration in the glomeruli. The filtration would therefore become easier, and increased formation of urine would continue until all the excess of fluid had been removed again. But suppose that to the saline we add some colloid which will not pass through the glomerular membrane and which will exert an osmotic pressure about equal to that exerted by the plasma proteins, then on injection of this mixture there will be no diminution of the force opposing the filtration, and therefore no increased formation of urine. The injected fluid will therefore remain in the blood vessels and so will assist in maintaining the blood pressure and the necessary circulation of the blood corpuscles for a considerable time. A suitable non-toxic colloid for the purpose is furnished by gum arabic; so that **gum-saline** injections have found extensive applications in combating the circulatory disturbances caused by wounds. We mentioned that the osmotic pressure of the proteins of the plasma amounted to 30–40 mm. Hg. The osmotic pressure of the gum in the injection fluid should also have this value if the normal conditions are to be reproduced as accurately as possible.

In concluding this account of osmotic pressure we wish to point out that these values of osmotic pressure are much smaller than those observed in the case of solutions of crystalloid substances such as sugars and salts. This is because the osmotic pressure exerted by any substance in solution is practically proportional to the number of molecules of it present per unit volume of the solution, but is independent of their size or kind. In the case of

proteins and gum where the molecules are large there will be but relatively few of them in unit volume of solutions of ordinary strengths, therefore the osmotic pressure exerted by these will be low. On the other hand since salt molecules and sugar molecules are comparatively small, even a small weight of any of these substances will be made up of a large number of molecules, so that their solutions will contain a relatively great concentration of molecules, and so will exhibit a high osmotic pressure. As a matter of fact, a substance in solution behaves just as if it were a gas occupying the same volume as the volume of the solution. The osmotic pressure exerted by it in the solution is identical with that which it would exert if it were converted into a gas occupying a volume equal to that of the solution and at the same temperature. Now a gram molecule of any gaseous substance which is made to occupy a volume of 22·4 litres exerts a pressure of just one atmosphere at 0°C. Similarly a gram molecule of any substance dissolved in a volume of 22·4 litres of solution exerts an osmotic pressure of one atmosphere at 0°C. In other words a solution containing one gram-molecule in a litre will exert an osmotic pressure of 22·4 atmospheres at 0°C. since the osmotic pressure is proportional to the concentration. In the case of an electrolyte, however, the value will be even higher than this because the splitting up of some of the molecules into their ions leads to an increase in the total number of particles in solution, and an ion is just as capable of producing its share of the osmotic pressure as is a whole molecule.

We have mentioned that the freezing point of a solution is depressed below that of the pure solvent by an amount, which, like the osmotic pressure, is a measure of the degree of attraction between the molecules of the dissolved solid

and the solvent. Also, as in the case of the osmotic pressure, the depression of the freezing point is dependent upon the total molecular concentration of the solution. For this reason the determination of the freezing point of urine is a valuable means of deciding whether or not the urine is abnormally dilute or abnormally concentrated. Of course such a determination will tell us nothing as to the particular kind of molecule present in excess or in subnormal amount, for, at least in dilute solutions, the depression of the freezing point and the osmotic pressure depend only upon the total concentration of molecules and ions present and not on their kind.

It is often found difficult to realise the relation between the process of diffusion of a dissolved substance throughout a mass of solvent and the osmotic pressure it exerts when confined by a semi-permeable membrane. Possibly the matter may be made clearer by the following consideration. The fact that a dissolved solid can diffuse throughout a mass of liquid in which it is soluble is due to the same attraction between solute and solvent which we found to be shown by the existence of osmotic pressure. In the absence of any membrane, not only the solvent molecules but also the molecules of dissolved solid are free to move in response to this attraction. They therefore diffuse out among the solvent molecules, until equality of concentration is attained throughout the bulk of the solution. The system then remains in equilibrium, its free energy having become a minimum. On the other hand, when the dissolved solid is held inside a membrane, through which it cannot diffuse, the attraction between it and the molecules of its solvent can lead to the movement of solvent molecules only. These are drawn inside the membrane, with the result that an increased pressure—the osmotic

pressure—is set up. It is important to realise that the tendency to equalisation of concentration of a dissolved substance will be manifested in the body fluids of organisms, and that any process in which this tendency is resisted, by which differences of concentration are produced in a solution, necessarily requires the expenditure of energy in the part of the tissue which brings about the change. Of course, in the organism, processes of diffusion are often accelerated by osmotic effects. For, if we have a case where a solution of a crystalloid is separated, by a membrane through which it can readily pass, from a solution of a colloid which will not pass through the membrane, then as the water is drawn by osmotic attraction into the colloidal solution, the dissolved crystalloids will be so drawn in as well. But if the crystalloids are at all hindered in their diffusion by the membrane, then they will not be absorbed so readily as the water in which they are dissolved. These considerations are important in connection with the discussion of the mechanism of absorption from the alimentary canal, where the digested food represents the solution of crystalloids separated by the intestinal epithelium from the solution of colloids—the blood plasma—in the capillaries of the villi.

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CHAPTER XIX.

FURTHER APPLICATIONS OF PHYSICAL CHEMISTRY: COLLOIDS: ADSORPTION: THE REACTION OF BODY FLUIDS: THE FUNCTIONAL IMPORTANCE OF ELECTROLYTES.

Colloids: Adsorption.

> "The colloid possesses ENERGIA. It may be looked upon as the probable primary source of the force appearing in the phenomena of vitality."—*Graham.*

It is usual to regard a solution of a simple crystalline substance such as urea or glucose as representing the simplest type of solution with which we are acquainted. One imagines the molecules of the dissolved substance to be distributed singly between those of the water or other solvent, and to be in constant movement like the molecules of a gas. In the case of dissolved salts the system is more complex because the salt will in general be split up to a considerable extent into its component ions. But also in this case the dissolved molecules are small and active. But when one comes to consider a protein or a starch it is easy to see that a solution of such a substance differs very much from one of urea or of a salt, inasmuch as the ultimate particles of a dissolved protein are no longer small and active, but large and therefore sluggish. For this reason they diffuse through water at a rate which is very small compared with that at which the crystalline substances will travel. This observation was first made by Graham, who classified all soluble substances into two classes—those which were characterised by the property of rapid diffusion and those which lacked

this property. Most of the members of the first group are crystalline substances; Graham therefore called these the **crystalloids.** On the other hand he invented the name **colloids** for the slowly-moving substances, after the Greek, for a typical member of this group, namely glue (Gr. κολλα= glue). He found, also, that the substances included in his group of colloids were not capable of passing through a membrane of parchment which offered little or no obstacle to the passage of crystalloids such as sugars and salts. In a general kind of way it can be said that the reason why a colloid in solution will not diffuse through a membrane is that its molecules are too large to pass through the spaces between the molecules of the substance of the membrane. This has an important bearing on the significance of the process of digestion, for the great bulk of the soluble constituents of ordinary foods—proteins, starches, for example—are colloidal in nature, and so, therefore, would not pass at all readily by a process of diffusion through the layers of the mucous membrane separating the cavity of the small intestine from the blood vessels. But during the changes which occur in digestion the large molecules of these colloids are broken down, as we have seen, into simpler molecules—amino-acids, sugars—all of which are members of the crystalloid group, and so are characterised by rapid diffusion. From this point of view we may say that the result of digestion is to convert the colloidal constituents of the food into simpler crystalloid products to which animal membranes are permeable.

Of these crystalloids some, at least, are resynthesised to form the colloidal constituents of the cells and tissues of the body—the sugars to form glycogen and the amino-acids to form proteins. But it will be realised from what

we have already said on a previous page (39) that the advantage of this digestive breakdown of the food to the crystalloid condition is not merely that it facilitates absorption of the products from the intestine, but so that it provides an opportunity for the recombination of these products in proportions and arrangements differing widely from those which originally existed in the food. So that from vegetable starch, animal starch or glycogen can be formed; from the proteins of mutton, those of man.

The slight diffusibility of colloids is, however, by no means the only general property which these substances possess by reason of the large size of their molecules. Many of their further properties are due to the circumstance that the possession of such large molecules leads to the exposure of an enormous total surface of the colloidal substance to the liquid through which it is dispersed—they are, therefore, surface effects. In describing the properties of the bile salts we explained briefly the nature of surface energy (p. 99). Now although they possess the property in a very marked degree, the bile salts are not the only substances which have the effect of lowering the energy of a liquid surface. Many other dissolved solids bring about the same result, and the more of such a substance is present in solution the greater the lowering of the surface tension. Now in all processes involving energy it is a matter of universal observation that the free energy of any system tends to become a minimum, and that if any change in the system will produce a reduction of the free energy, then that change tends to take place. For example, a mass of matter tends to lose its free energy by falling from an initial position to a point nearer the earth; heat tends to flow from hot to cold bodies so that equality of temperature is established, and the heat

energy, although undiminished in amount, is no longer available for conversion into mechanical work—and so on. If now we consider the case of a solution of a substance whose property it is to lower surface tension, it will be seen that if we start with the dissolved solid uniformly distributed throughout the mass of liquid, we could diminish the free energy of such a system by causing the dissolved solid to leave the main bulk of the solution, and to be accumulated as much as possible in the surface layers, where it produces its lowering of surface tension. As a matter of fact, such a change would occur spontaneously; it is indeed found that any substance which reduces surface energy exists in greater concentration in the surface layers of a liquid than in the main bulk of it. The dissolved solid diffuses into the surface layers until the diminution of surface tension it produces is just balanced by its tendency to diffuse back into the main bulk of liquid where its concentration is less. This process of surface concentration of a dissolved substance is called **adsorption.** It is to be noted that it is merely a physical union between the dissolved substance and the surface, and it does not occur in those same definite ratios in which chemical union always takes place. Naturally, the amount of solute adsorbed depends upon the total area of adsorbing surface, increasing very markedly as the surface is increased, until quite striking cases of retention of a solid are observed if the surface be large. This can be illustrated quite easily with potassium permanganate. This salt lowers the surface energy at a surface of contact between glass and water, and therefore tends to become concentrated at such a surface. But the effect is too small to enable one to detect any increased depth of colour at the sides of a glass beaker containing

permanganate solution. But if we expose the solution to an enormous area of glass by allowing it to trickle through a tube packed with glass wool, then the permanganate is adsorbed to such an extent that the liquid which drops from the lower end of the tube is practically colourless.

The importance of this process of surface concentration from the point of view of the properties of colloids is that the particles of a substance in colloidal solution present an enormous total area of surface to the solution, so that there is abundant opportunity for adsorption of dissolved solids on to the colloidal particles. It may help the student to realise what an enormous area is available for adsorption in colloidal solutions if we mention that if a sphere of material of about the size of a tennis ball were to be subdivided into particles of the size ordinarily found in colloid solutions, the total of the areas of these particles would amount to twenty times the area of a tennis court!

It might be of interest to mention that the relation which has been found to exist between the concentration of a solution and the amount of dissolved solid adsorbed on a certain area of adsorbing surface is that the concentration of adsorbed substance is proportional to the square or some near root of the concentration left behind in the bulk of the solution. So that if c_a represents the concentration of substance adsorbed and c_s the concentration of the substance left in the solution, then

$$c_a = k\sqrt{c_s}$$

where n is a number approximately equal to 2, and k is a constant which varies according to the particular substances and surfaces used.

Expressed in words, this mathematical expression means that if the concentration of dissolved solid is doubled, then the concentration of adsorbed solid is multiplied not by 2 but only by a root—the *n*th root of 2, and so on. So that the ratio

$$\frac{\text{adsorbed solid}}{\text{dissolved solid}}$$

becomes smaller as the total concentration of the solid is increased. A greater *proportion* of solid will therefore be adsorbed from a dilute than from a stronger solution;

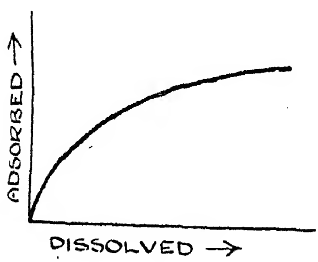

FIG. 20. Diagram of the usual form of curve which expresses the relation between the amount of a substance adsorbed on to a surface and the concentration of it in the bulk of solution. At low concentrations a greater fraction of the total dissolved solid is adsorbed than at high concentrations.

indeed, as the concentration becomes large, the amount of adsorbed substance tends to increase more and more slowly, until the surface becomes almost completely saturated, further addition of solute leading then to comparatively little further adsorption. This can easily be confirmed by substituting various values of c_s in the formula we have just given, and having decided on values of k and n, calculating corresponding values of c_a, the amount of solute adsorbed. On plotting the results of such a calculation it will be at once seen that the amount of adsorbed solid is expressed by a curve which increases more and more slowly as the concentration is increased (see Fig. 20).

On account of the occurrence of this process of adsorption we shall not expect to find anything approaching an uniform distribution of the salts and sugars and other crystalloids present in cell protoplasm, in which so large a proportion of the constituents are colloidal in nature. This is a result of fundamental importance in the study of the chemical mechanics of the cell.

Further, this process of adsorption is of importance in the study of the mechanism of enzyme action. In dealing with enzymes we have already pointed out (p. 173) that probably the first step in the process whereby an enzyme accelerates a chemical change consists in a condensation of the reacting substances on the surfaces of the colloidal enzyme particles. Evidence in support of this view is obtained from a study of the rate at which an enzyme action proceeds under various conditions of concentration (p. 173), and also from the fact that it is possible to make colloidal solutions of such inert materials as platinum and gold which show catalytic properties similar in some respects to those of enzymes. A colloidal solution of platinum can catalyse the hydrolysis of an ester in a way which resembles superficially the action of a lipase, and doubtless this resemblance is the expression of the fact that in both cases surface forces are playing an important part in the process.

As we have come across these colloidal solutions of the metals during the course of our discussion we might mention some further details with respect to them. They are prepared either by precipitating the metal chemically—by means of a reducing agent—from a dilute solution of one of its salts or by forming an electric arc between two electrodes made of the particular metal desired and immersed in pure water. In this second case

the metal is probably vapourised in the intense heat of the arc itself, and the vapour is immediately condensed in minute particles when it comes into contact with the cold water. In this way an exceedingly fine suspension of metallic particles is produced which possesses the typical properties of colloidal solutions in general, inasmuch as these properties depend primarily on the presence of a solid, so dispersed through the liquid phase as to present an enormous boundary surface of contact between the two. Of course the large size of the platinum particles in such a colloidal solution of the metal, when compared with that of ordinary salt and sugar molecules, is not due to the fact that the individual platinum molecules are large, but that each particle in the solution consists of an aggregate of a number of metallic molecules. It should be mentioned that the metallic particles are sufficiently large to catch light which falls upon them, and to scatter it sideways. Hence if the colloidal solution is illuminated by rays passing in a more or less horizontal direction, the scattered light will pass up the tube of a microscope, and each colloidal particle will appear as a bright point under suitable magnification. An arrangement for observing this effect is termed an **ultra-microscope.**

There is one important respect in which the colloidal solutions of proteins and starches differ from those of platinum and gold and of other inorganic materials which have been obtained in the colloidal or dispersed condition. This lies in the intimacy of the relationship between the colloidal particles themselves and the water in which they are suspended. For while the particles of metal in a platinum "sol" are more or less independent of the water molecules, so that the system as a whole forms a

thin liquid like water itself, the molecules of a dissolved protein seem to be combined in some way with a considerable amount of water, so that the solution is sticky and viscous and readily forms a jelly-like structure or "gel."

An extremely important character common to both of these classes of colloids is the possession of an electric charge on the particles of which it is composed. This readily manifests itself by the movement of the particles which takes place when an electric current is passed through such a colloidal solution. In many cases it is easy to understand how this charge is acquired. For example, in the case of a platinum solution doubtless the particles send into the water platinum ions, which, being metallic ions, carry a positive charge, and so leave the remaining metallic particle negatively charged. A protein molecule in slightly alkaline solution acts as a weak acid, and therefore tends to send off positively charged hydrogen ions into the solution. On the other hand, in acid solution a protein acts on a base—resembling in this way the amphoteric amino-acids of which it is composed—and so tends to split off negatively charged hydroxyl ions so that the remaining bulk of the molecule forms a positively charged ion. It is evident that at a certain intermediate reaction a given protein will tend to give off both hydrogen and hydroxyl ions to equal extents; the remaining bulk of the molecule will then possess equal positive and negative charges, that is, it remains neutral. The reaction at which this occurs is said to be the **isoelectric point** of the particular protein (or other amphoteric substance), because at this reaction the protein molecules are not electrically charged, and therefore do not move in an electric field. We see then that either positively or

negatively charged colloids may exist, the charge determining the direction in which they tend to move when they are submitted to an electric current. It is the presence of this charge which renders the colloidal solution stable and prevents the suspended colloidal particles or molecules from coming together and adhering to form larger particles which would separate out of the solution. For the particles, possessing charges of similar sign, repel one another, and so are kept apart. If, however, there be added to a colloidal solution ions which carry a charge of sign opposite to that carried by the colloidal particles, then when such an ion meets a colloidal particle their charges will neutralise each other at least partially, and if a sufficient number of these discharging ions are present, all the colloidal particles lose their charges, and so can adhere together into larger masses, which settle out of the solution. Naturally the discharging power of an ion depends on the amount of charge it carries, and for that reason divalent ions have a much more powerful precipitating action than monovalent ions possess, while trivalent ions will act even in minute concentrations on account of the three units of charge which each carries. Of course, a given colloid will be precipitated only by ions which carry charges opposite in sign to its own.

From what we have said it will be easy to understand how it is that proteins are most readily precipitated when they are in the isoelectric condition, for then there is the maximum number of uncharged particles in solution so that aggregation can most readily occur.

It will be appropriate to finish this account of the general properties of colloids by reminding the reader of the various chief ways in which proteins can be thrown

out of solution. We have just mentioned that they can be precipitated by means of appropriately charged ions which may act in small concentrations. They can also be "salted out" of solution by means of a large amount of a neutral crystalloid such as ammonium sulphate, which causes the protein to separate merely by attracting the solvent water from it. Lastly, many proteins may be removed from solution in the form of a coagulum by heating their solutions under suitable conditions or by adding alcohol to them. But in these cases the protein is not recovered unaltered, for the process of coagulation is preceded by a chemical decomposition (p. 24).

The Reaction of Body Fluids.

"The heart knoweth his own bitterness."—*The Wisdom of Solomon.*

It has been known for a long time that many physiological processes are very profoundly influenced by the reaction of the medium in which they take place. Take, for example, the heart; if the medium which is surrounding it or the fluid which it contains, is too alkaline, then it comes to rest usually in a contracted condition. On the other hand, if the fluid surrounding the heart is too acid, then the beats again cease, leaving the musculature in a completely relaxed condition. But if the fluid supplied to it is of just the right reaction, the heart will go on beating continuously, alternately relaxing and contracting. The important point to notice is that there is just a very narrow range of reaction over which the heart muscle can function; outside the limits of that narrow range its rhythmic activity cannot be maintained. This is one of the best examples we can choose of the very profound influence of small changes of reaction on the physiological behaviour of a tissue.

From this it will be easy to understand the reason why, in physiological work, it is necessary so frequently to take account of the reaction of the fluids which are being used; for not only the heart beat, but also many other physiological processes—including such as ferment actions, the development of eggs; in fact, practically all tissue activities can occur only in a medium possessing a certain reaction, and are arrested in media, which are appreciably more acid or more alkaline. Of recent years, knowledge of the reaction of solutions has been made very much more definite than it formerly was, because it has now been realised that an acid solution owes its typical properties to the presence of excess of hydrogen ions, and an alkaline solution owes its alkaline properties to the presence of excess of hydroxyl ions, while a neutral solution is one which contains equal numbers of hydrogen and hydroxyl ions. Now the typical neutral liquid is water. Pure water is not absolutely free from either hydrogen or hydroxyl ions, but it contains these ions in equal concentrations, for these ions arise by the electrolytic dissociation, or, as we say, the ionisation of a certain number of the water molecules. One can represent the process by an equation as follows:—

$$H_2O \rightleftarrows \overset{+}{H} + \overset{-}{OH}$$

Now it is evident that by the ionisation of water there must necessarily be produced equal numbers of hydrogen and hydroxyl ions, simply because when a hydrogen ion has been split off from the water molecule, a hydroxyl ion is all that remains.

Suppose we wish to increase the concentration of hydrogen ions in a solution above the concentration of

hydroxyl ions, the simplest plan is to add an acid, for example, hydrochloric acid; when this is dissolved in water its molecules ionise, yielding hydrogen ions and chlorine ions, the hydrogen ions as before being positively charged, and the chlorine ions being negatively charged. These hydrogen ions from the acid are added to the hydrogen ions from the water molecules, giving an increase of hydrogen ions over and above the concentration of hydroxyl ions, which have come entirely from the water molecules. On the other hand, suppose we wish to increase the concentration of hydroxyl ions in a solution; suppose, that is to say, we wish to make the fluid alkaline, then there must be dissolved in it an alkali, such as caustic soda. This will ionise into sodium ions, which are charged positively, and hydroxyl ions, which are negatively charged. Thus there will be present in a solution of caustic soda the excess of hydroxyl ions derived from the soda itself, together with the hydroxyl ions resulting from the ionisation of the water; there will also be those hydrogen ions, just a very small quantity of them—which have arisen also from the water.

It is very important to realise that every aqueous solution, even the most alkaline, still contains a certain small quantity of hydrogen ions. Hydrogen ions are not confined to solutions of acids. A solution is alkaline because it contains a large excess of hydroxyl ions, and if it be an aqueous solution it necessarily must contain some hydrogen ions because water always ionises to a small extent, giving hydrogen and hydroxyl ions; similarly, in a solution of an acid, even of the strongest acid, there are just a few hydroxyl ions which arise from the water present on account of the tendency of the water molecules to ionise.

An acid solution can never be free from hydroxyl

ions, nor that an alkaline solution is free from hydrogen ions. But it must be pointed out that this ionisation of water is a balanced reaction, so that when an excess of any product of that reaction, such, for example, as hydrogen ions—when an excess of these ions is added to the system, then the reaction tends to go in the backward direction, for some of the excess hydrogen ions will combine with some of the hydroxyl ions to form water molecules. So that in an acid solution, containing an excess of hydrogen ions, although there will always be some hydroxyl ions present, there will be a less concentration of these hydroxyl ions than in pure water. On the other hand, if an excess of hydroxyl ions be added to this system, then some of these excess hydroxyl ions will combine with some of the hydrogen ions which have been obtained from the water molecules, so that there will be less hydrogen ions in the alkaline solution than there are in pure water. But there will always be some hydrogen ions in the alkaline solution.

We can express these relationships much more exactly, for the law of mass action applies to a reversible equilibrium such as ionisation of water. The law of mass action states that the products of the concentrations of all the reacting molecules on one side of the equation bears a constant ratio to the products of the concentrations of the reacting molecules which are formed in the reaction. This is true of all chemical equilibria. If we apply it to our particular case we conclude that the product of the concentration of hydrogen ions, and of the concentration of hydroxyl ions bears a constant ratio—let us call it k—to the concentration of the water molecules in any system where these three constituents occur together. The concentration of each substance is measured in

gram-molecules per litre, so that by the concentration of hydrogen ions is meant the number of gram-molecules of hydrogen ions per litre of the solution. A solution of unit concentration of hydrogen ions—a so-called "normal" solution—will contain one gram-molecule of hydrogen ions per litre. And since the gram-molecular weight of a hydrogen ion is 1, normal concentration of hydrogen ions will be 1 gram per litre. On the other hand, the normal concentration of hydroxyl ions will be 17 grams per litre, for the gram-molecular weight of a hydroxyl ion is 17. ($O=16+H=1$.) If now we write the concentration of hydrogen ions as $[\overset{+}{H}]$, the concentration of hydroxyl ions as $[\overset{-}{OH}]$, and that of the water molecules as $[H_2O]$, we can represent the application of the law of mass action to the ionisation of water by the equation:—

$$[\overset{+}{H}] \times [\overset{-}{OH}] = k\,[H_2O],$$

Now we shall see immediately that the actual number of water molecules ionising in any solution is an extremely small fraction of all that are present. This is true even in the case of pure water or a neutral solution, and when acid or alkali is added the ionisation is still further depressed. It is, therefore, true to say that in spite of any changes which may occur in the degree of ionisation, the total number of dissociated water molecules is so small that they may be neglected in comparison with the relatively enormous concentration of undissociated water molecules. In other words, we are justified in overlooking the fact that each change in reaction involves either the formation or the ionisation of a few water molecules, and so can count the concentration of

undissociated molecules of water as an unvarying quantity. We will call it *c*, so that our factor $[H_2O]$ is replaceable by *c*; then

$$[\overset{+}{H}] \times [\overset{-}{OH}] = kc.$$

Since both k and c are constant quantities their product is also a constant, so that instead of writing *kc* we can write one large K to stand for the two constants multiplied together. Then

$$[H] \times [\overset{-}{OH}] = K.$$

This K is called the ionisation constant of water.

About this last equation there are two important things to say. One is that it applies to any system whatever which contains water and hydrogen ions and hydroxyl ions. This will include pure water itself, and, as we have said, all aqueous solutions. In an acid solution the concentration of hydrogen ions will be large. But the product of the hydrogen ion concentration and the hydroxyl ion concentration is necessarily constant, as we have seen, therefore the hydroxyl ion concentration must be correspondingly small. On the other hand, in an alkaline solution the hydroxyl ion concentration is large. The product of hydroxyl and hydrogen ion concentrations is always the same constant, K, therefore the hydrogen ion concentration in an alkaline solution must be correspondingly small. This is merely a quantitative mathematical statement of what we have already learnt about solutions of hydrochloric acid and caustic soda. In all solutions—acid, alkaline and neutral—if the concentration of hydrogen ions be doubled the concentration of hydroxyl ions becomes halved, for one half of them combine to form water with some of the excess hydrogen

ions. The product of their concentrations thus remains constant.

The other important topic connected with this equation is the value of K. In pure water we have already seen that the hydrogen ion concentration is necessarily equal to the hydroxyl ion concentration. That means to say, then, that for pure water $[\overset{-}{OH}]=[\overset{+}{H}]$, so that the equation can be written

$$[\overset{+}{H}]^2=K.$$

So K becomes equal to the square of the concentration of hydrogen (or hydroxyl) ions. This is true only for the particular case of pure water or of a perfectly neutral solution. Now if we have some means whereby we can analyse water, and find out what is the concentration of hydrogen ions in it, then we shall be able to determine this value of K; and such methods of analysis have been devised. By physico-chemical methods it is possible to determine the hydrogen ion concentration of pure water, and it is found to be one ten-millionth of normal, that is to say, in a litre of pure water there is only one ten-millionth of a gram of hydrogen ions. It is more convenient to represent this not as a fraction, but as 10^{-7}, which means one ten-millionth, for $10^{-7}=\frac{1}{10^7}$, which is $\frac{1}{10,000,000}$. The hydrogen ion concentration of pure water, or of a neutral solution, is then $10^{-7}\times N$. It therefore follows that our constant K—the ionisation constant for water—is equal to $[10^{-7}]^2$, i.e. 10^{-14}. This means to say that in every aqueous solution the product of the hydrogen ion concentration and the hydroxyl ion concentration is always equal to 10^{-14}.

The hydrogen ion concentration in pure water or in a

neutral solution is $10^{-7} \times N$. It will be seen, therefore, that in speaking of the hydrogen ion concentrations of fluids whose reactions are near the neutral point—and this is the case with most biological fluids—we have to deal with very small fractions—and it has been found convenient, for biological work at any rate, to describe these hydrogen ion concentrations by a rather special nomenclature. Instead of naming the actual concentration of hydrogen ions it is simpler, from some points of view, to name only the index—the power or potence of 10—which is required to express the concentration. An index or power used in this way is termed a p_H. Instead, then, of saying that the hydrogen ion concentration in a certain solution is 10^{-7}, it is usual to say that the solution has a p_H of 7. It is to be noted that the negative sign of the index is omitted in the p_H value. In other words, the p_H tells us the negative value of the power to which 10 must be raised in order to give the actual concentration of hydrogen ions in gram-molecules per litre. Conversely, therefore, a solution of $p_H 6{\cdot}5$ has a concentration of hydrogen ions of $10^{-6.5}$ grams per litre, and so on. But here a little confusion is liable to arise. A solution of hydrogen ion concentration 10^{-7} has a p_H of 7, as we have said. Now a solution of hydrogen ion concentration 10^{-6}, that is one-millionth of a gram-molecule per litre, has of course 10 times the concentration of hydrogen ions that the first solution of $p_H 7$ possessed, but according to our scheme the p_H of the second more acid solution must be written as 6. In other words, then, as the hydrogen ion concentration increases, the p_H number diminishes, in such a way that every diminution of unity in the p_H means an increase of hydrogen ion concentration to ten times the former value, so that a

solution of $p_H 6$ has ten times the hydrogen ion concentration of a solution of $p_H 7$ and a solution of $p_H 5$ has ten times the hydrogen ion concentration of a solution of $p_H 6$.

Now we said that a neutral solution has a p_H of 7. Solutions with p_H's smaller than 7 will be acid solutions, and solutions with p_H's greater than 7 will then be alkaline solutions. So that from a slightly different point of view we can say that the larger the p_H number, the more alkaline the solution is. There might be considerable possibility of confusion in this nomenclature if one were dealing with a wide range of p_H's, but biological fluids do not differ very much with regard to their p_H's—they are all neutral or nearly neutral—so that we have to deal with a comparatively limited range of reaction. Indeed, we noticed at the outset that life processes were only possible within a certain limited range of reaction. It must always be clearly realised that the p_H scale is a logarithmic scale, so that $p_H 6{\cdot}5$ does not represent a concentration of hydrogen ions which is arithmetically half way between those represented by $p_H 7$ and $p_H 6$ respectively.

Now that we have explained how important it is to know the p_H of any solution which is being used in biological experiments, we ought to say something as to how p_H's are measured. This measurement is most easily carried out by the use of indicators, and of certain solutions of standard p_H, which are known as **buffer solutions.** A buffer solution is one to which there may be added either acid or alkali without producing very much change in acidity or alkalinity. Sodium phosphate is one of the commonest of these buffer substances. They are called buffer substances because, like a buffer, they ease the shock of the addition of acid or alkali, and

prevent large changes of hydrogen ion concentration, even when strong acids or bases are added. If, for example, hydrochloric acid be added to a solution of ordinary sodium phosphate, the acid reacts with the salt and yields sodium di-hydrogen phosphate and sodium chloride:—

$$Na_2HPO_4 + HCl \longrightarrow NaH_2PO_4 + NaCl.$$

Now sodium phosphate itself is an almost neutral substance, and so are the sodium di-hydrogen phosphate and the sodium chloride which are formed as a result of the addition of the acid. In other words, after the addition of acid to an almost neutral solution, we end up with substances which themselves are still practically neutral. Of course it might have been said that we could prevent a solution from becoming acid when an acid is added to it, by having present first of all some alkali, but in this case a very large change in the reaction of the whole solution from alkaline to neutral would have been produced. We should have started with an alkaline solution with a high p_H number and perhaps have ended up with a nearly neutral solution of p_H about 7, so that the change of p_H would have been great in this case. On the contrary, if we used a phosphate or other buffer we may start with a solution whose p_H is less than 8, and finish with a solution whose p_H is still greater than 7, in spite of the addition of a considerable quantity of acid to the solution.

Buffer solutions are useful in two ways. In the first place, suppose that some biological process is occurring, which leads to the formation of acid products,—it often happens that these acid products by their own acidity stop the process by which they are being liberated.

A ferment action, for example, is a case in point. A ferment is working and liberating acid products. Now these acid products will destroy the ferment by their acidity, and so bring its activity to an end unless there is present in the solution some buffer which will take up the acid formed, and prevent the solution from changing its reaction. Thus, by means of a buffer solution, it is possible to keep a reacting mixture at almost constant p_H.

Then, secondly, because these buffer solutions do not readily change their p_H when acid or alkali is added to them, they are useful as standards of p_H, because they will not be easily affected by carbonic acid absorbed from the air or by alkali dissolved from the glass of the bottle in which they are kept. Of course the p_H's of these standard buffer solutions have to be measured initially. This involves a rather complicated electrical method which we cannot describe here, but once the series of standards has been obtained the p_H of, say, a biological fluid can be measured by comparison with these standards by means of indicators.

The principle of the method of using indicators is easily understood. Each indicator has a certain narrow range of p_H over which it changes colour; but the p_H at which one indicator changes colour is in general different from that at which a second changes its colour. Let us take litmus as our example. In solutions more acid than a certain p_H it is red, in solutions more alkaline than this p_H it is blue, but over a certain narrow range of p_H it is a mixture of red and blue, that is purple. Now the more alkaline the solution the bluer it is, the more acid the solution the redder the litmus is, and so the p_H of any unknown solution can be determined by adding litmus to it, and then matching it against a series of standard p_H

solutions to which litmus has also been added. For if in any standard solution the tint of the litmus is the same as in the unknown solution, then the p_H in the unknown is the same as that in the standard. Of course, litmus can only be used over a certain range of p_H when its colour is changing from red to blue—when it is purple. Over any other p_H range it will either be all too red or too blue for the small differences of shade to be distinguished easily: but for other ranges of p_H there are other indicators available. Litmus changes its colour at a certain p_H, methyl orange will change at a different p_H from red to yellow, and phenolphthalein at a still different p_H from colourless to crimson. It is very important to realise that the p_H at which a given indicator shows its neutral colour is not necessarily at the point of true neutral reaction, and that any given indicator can be used only over the range when it is changing colour rapidly, because it is sensitive enough only over that particular range. Really what we have described is not exactly the measurement of p_H, but rather the comparison of the p_H's of unknown fluids with known standards; and this comparison is usually carried out in a comparator which allows of the correction for any colour which there may be originally in the solution under examination before the indicator is added by arranging in front of the colourless standard solution, a tube of the unknown fluid through which the standard is viewed while the matching of the tints is being carried out.

Perhaps the measurements of hydrogen in concentration most important from the physiological point of view are those which are carried out on blood. We shall illustrate this by one or two points. In the first place, the measurement of the hydrogen ion concentration

of the blood of a patient gives a sure test for the presence of strong harmful acids which are liable to arise during the course of abnormal or disordered metabolism. For example, the aceto-acetic and β-oxybutyric acids formed in diabetes produce an increase of the concentration of hydrogen ions in the blood.

Then also the measurements of the hydrogen ion concentration in blood gives us a clue to the state of combination of its carbon dioxide. As the discussion of this topic gives an interesting and clear insight into the mechanism of buffer action, we shall deal with it in brief outline. If we dissolve carbon dioxide in water, the carbonic acid which is formed splits up to a certain extent into hydrogen and bicarbonate ions, thus:—

$$H_2CO_3 \rightleftarrows \overset{+}{H} + \overset{-}{HCO_3}$$

The solution is therefore acid owing to the presence of these hydrogen ions formed from the H_2CO_3 molecules (together, of course, with the small concentration of hydrogen ions obtained by the ionisation of the water molecules themselves, which we can neglect for the sake of simplicity). To take a definite instance—suppose we dissolve in water as much carbon dioxide as it will take up when exposed to a tension of 40 mm. Hg. of the gas at body temperature the solution is found to have a reaction of p_H=about 4. If now we dissolve this same amount of carbon dioxide not in pure water, but in a solution which already contains an abundance of bicarbonate ions, then we may say that the space available for the bicarbonate ions which tend to be formed from the H_2CO_3 molecules is already to a great extent occupied. The carbonic acid therefore cannot split off its $\overset{-}{HCO_3}$ ions to

such a large extent as it could in pure water, and, seeing that in the case of this acid the formation of a bicarbonate ion is a necessary accompaniment of the formation of a hydrogen ion it follows also that less hydrogen ions can be formed. In other words, in this case the solution becomes less acid when exposed to the same tension of carbon dioxide than pure water under the same conditions. Now we can easily obtain a solution containing an abundance of $\overline{H}CO_3$ ions by dissolving sodium bicarbonate in water. This salt ionises into sodium and bicarbonate ions as follows:—

$$NaHCO_3 \rightleftarrows \overset{+}{Na} + \overline{H}CO_3.$$

Therefore a solution of sodium bicarbonate depresses the ionisation of carbonic acid, and is more alkaline than pure water when both are exposed to a given tension of carbon dioxide. And, of course, the degree of depression of the ionisation of the carbonic acid depends on the concentration of bicarbonate ion previously present in the solution, and this in turn on the strength of the bicarbonate solution itself. If we take a solution of bicarbonate such as that on acidification it gives off, say, 50 c.c. of carbon dioxide per 100 c.c. of the solution, we then find that the p_H of this solution at 40 mm. Hg. CO_2 tension is 7. The student who has followed our discussion of the p_H nomenclature will at once realise that this means that the hydrogen ion concentration in this solution bears the ratio 10^{-7} to 10^{-4} to that in the pure water exposed to 40 mm. Hg. CO_2 tension. In other words, the concentration of hydrogen ions in this bicarbonate solution is 10^{-3} or $\frac{1}{10^3}$, i.e. one-thousandth of what it is in the pure aqueous solution of carbon

dioxide, so great is the depressing action of the bicarbonate on the ionisation of the carbonic acid. It will now be clear what we mean when we say that sodium bicarbonate is a buffer towards carbonic acid; it simply prevents to a very large extent the ionisation and the liberation of its hydrogen ions.

Now we have chosen this particular strength of sodium bicarbonate solution because it contains about the same total amount of combined carbon dioxide as normal blood at 40 mm. Hg. CO_2 tension, as can be seen from the dissociation curves given in Fig. 17, p. 215. If now we measure the hydrogen ion concentration of blood at 40 mm. Hg. carbon dioxide tension we obtain a p_H of 7·4—which is practically the same as that of the bicarbonate solution under the same conditions. We must therefore suppose that in the blood we have the same concentration of bicarbonate ions depressing the ionisation of carbonic acid as in the bicarbonate solution; in the blood we have also the same total amount of carbon dioxide combined as in the sodium bicarbonate solution. We conclude that when carbon dioxide is taken up by blood it goes into a form in which it is ionised to exactly the same extent as is sodium bicarbonate under the same condition; we believe therefore that the form in which the carbon dioxide is taken up is that of sodium bicarbonate itself. We have chosen a CO_2 tension of 40 mm. Hg. at which to compare the blood and the bicarbonate solution; but if we had used the data corresponding to any other tension of carbon dioxide we should have observed a similar correspondence of p_H, and so can draw the conclusion that at all tensions of carbon dioxide the whole of the combined carbon dioxide of blood is in the form of sodium bicarbonate.

We have incidentally referred to the powerful buffer action of sodium bicarbonate in depressing the ionisation of carbonic acid. This effect plays a very important part in the maintenance of the normal slightly alkaline reaction of the blood during the transport of carbon dioxide in the body. When for any reason the amount of sodium bicarbonate present in the blood is below normal this buffer effect is not so efficient, and the blood tends to become abnormally acid. This tendency, when it is observed in pathological conditions, is often corrected by the injection of sodium bicarbonate solutions into the blood or by the administration of considerable quantities of this salt by the mouth, in order that the tissues of the body shall not suffer by exposure to a fluid of inappropriate reaction.

The Functional Importance of Electrolytes.

"Senza sali nei liquidi periprotoplasmatici non è possibile la vita."
—*Bottazzi.*

We have already mentioned, in dealing with the question of the reaction of body fluids, that tissues and organs are very sensitive to changes in composition of the media by which they are bathed. And not only is this true with regard to the concentration of hydrogen ions, but also with respect to the other ions occurring in the solution. In particular, life processes in tissues are found to be dependent in a very special manner on the concentration of the metallic ions with whch they are in contact, and more particularly of the ions of sodium calcium and potassium.

This fundamental fact was discovered by Sidney Ringer in the early 1880's during a series of researches, the object of which was to determine the part played by the

various constituents of blood in the maintenance of the rhythmic contraction of the ventricle. By perfusing the frog's heart with solutions of various compositions he was able easily to show that while the proteins and other organic constituents of the blood could be omitted without affecting the activity of the heart musculature to any appreciable extent, any change of the amount of inorganic salts from the concentrations in which they occur normally in the blood led to marked abnormalities in the behaviour of the heart. For example, the complete withdrawal of all dissolved salts by perfusion with dis-

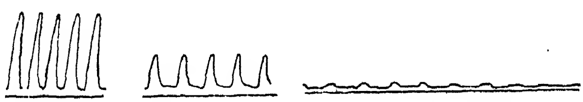

FIG. 21. A copy of Ringer's tracing showing the inability of a solution of pure sodium chloride to maintain the beats of the frog's ventricle.

The first beats were obtained 8 minutes after replacing the blood by ·75 per cent. sodium chloride solution.

The second series of beats was obtained after a further interval of 6 minutes and potassium were absent.

The third series was obtained 4 minutes later than the second.

tilled water at once brought the heart to rest, and destroyed the excitability of its musculature. Distilled water is therefore a poison to tissues. An isotonic solution of sodium chloride is little better than distilled water for the maintenance of the heart beat, for when perfused it causes the heart beat at once to begin to grow feeble, and finally to cease (Fig. 21); the saline solution is, however, somewhat more capable of preserving the irritability of the heart muscle, so that rhythmic beats once more return when a more suitable medium than plain saline is provided.

One of the most abundant inorganic constituents of the blood—apart from sodium—is calcium, and Ringer investigated the effect of adding small quantities of calcium chloride to the saline solution which he passed through the heart. As a matter of fact, he first observed the effect of calcium accidentally on an occasion when his saline solution had been made up erroneously with tap instead of with distilled water. He found that in a saline solution to which calcium had been added, the rhythmic beats of the heart continued for a time, but that gradually the relaxation of the heart became more and more delayed,

Fig. 22. A tracing (from Ringer) to show the effect of calcium salts in favouring the contracted condition of the ventricular muscle.

The first beats were obtained while the heart was perfused with blood.

The later groups of beats were obtained after the replacement of the blood by sodium chloride solution containing a little calcium.

until complete relaxation failed to occur before the succeeding systole. This tendency continued until the heart remained in a permanently contracted condition, and was incapable of relaxing at all (Fig. 22). Calcium, then, favours the contracted condition of the muscle at the expense of the relaxation.

Ringer next tried the effect of a saline solution containing a little potassium chloride, as this metal forms an important constituent of the normal blood. With such a mixture he found that the heart gradually came to rest in a completely relaxed condition. Having thus discovered the opposite tendencies produced by these

two metals he further investigated the effect of adding them both to the perfusion fluid. He then found that if the calcium were present in relatively large amount there was the characteristic tendency towards a permanent contraction of the heart, while if it was the potassium which preponderated, stoppage in a relaxed condition resulted. But if a certain balance between the amounts

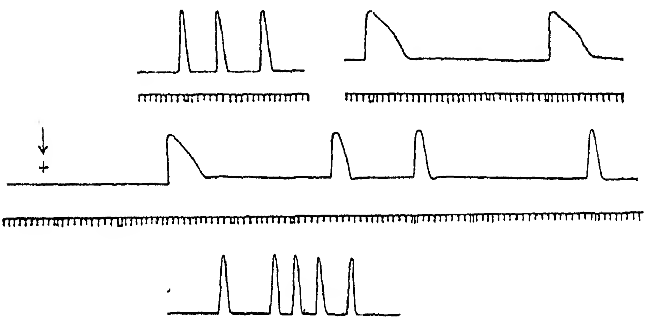

FIG. 23. The antagonism between calcium and potassium (Ringer).
The first recorded beats were obtained with the heart perfused with blood.
The second series was obtained with the heart perfused with a solution of sodium chloride containing calcium. The prolongation of the systole, which is typical of the action of calcium, is again seen.
At the arrow a small quantity of potassium chloride was added to the perfusion fluid. The effects of the calcium are antagonised and the beats become normal.
The last portion of the tracing was obtained 10 minutes after the addition of the potassium chloride.

of the two elements was hit upon, then the heart remained neither permanently contracted nor permanently relaxed, but contracted and relaxed alternately for a period of several hours, just as does a heart supplied with blood (Fig. 23). Indeed, this solution, containing, in addition to a basis of sodium chloride, small quantities of the chlorides of calcium and potassium, provides a practically normal environment not only for heart muscle,

but for living tissues in general, and is the fluid which is so extensively used in the laboratory under the name **Ringer's solution,** for the preservation of normal conditions in tissues and organs whose behaviour is being studied. Often a little sodium bicarbonate is added to the mixture in order to preserve a slight alkalinity, and so still further to imitate the conditions existing in the living body. From the remarks we have already made on the subject of the importance of the reaction of body fluids the student will readily appreciate the advantage of this addition.

Ringer was well aware that the effects he discovered were not produced only by the chlorides of potassium and calcium, but were common to all salts of these metals. At the time when he carried out his experiments, the theory of ionic dissociation had not been put forward. We see now that the effects are given not by the salts themselves, but by the positively charged calcium or potassium ions which are split off when the salts undergo electrolytic dissociation or ionisation in solution.

For the proper functioning of the heart, therefore, it is necessary that it should be bathed in a salt solution whose chief constituent is sodium chloride, but which must also contain calcium and potassium ions, and these in such proportions that their antagonistic tendencies are suitably balanced.

And what has been found to be true in the case of the heart has been found also to apply to many other tissues and organs. For the continued lashing of cilia, for the development of the eggs of marine organisms, and for the continued well-being of the animals which arise from them, the same balance of sodium, calcium and potassium ions is essential. And this is so whether we are

considering the external environment of an aquatic form or the internal environment furnished by the circulating blood of a land-dwelling beast. Disturb this ionic equilibrium but slightly, and abnormal and harmful effects at once manifest themselves. The delicate organism dies; the heart ceases to beat, and voluntary muscles which ordinarily remain quite quiescent except during moments of work, now show ceaseless unco-ordinated twitchings by which their energy is frittered away. This last fact is easily demonstrated by placing an excised muscle of the frog in pure sodium chloride solution. It soon commences to show small spontaneous twitchings, which gradually increase in vigour until the muscle begins to grow exhausted, and the movements die away. The addition of calcium salts tends to diminish this activity by reducing the irritability of the muscle; potassium salts increase the vigour of the twitches, and then finally poison the muscle and kill it. But in just the correctly balanced solution of all three salts the muscle will remain at rest, but nevertheless alive and normally irritable for days at a time. As Loeb says: "We owe it to the calcium and magnesium salts of our blood that our skeletal muscles do not contract rhythmically like our heart."

The student who has followed our discussions up to this point will now be in a position to realise the chief requirements of a fluid which is to be perfused through organs in order to maintain them in as normal a condition as possible when they are out of the body. It must possess a suitable osmotic pressure, so that it shall not damage the tissues by changing their water content; it must be of appropriate, usually slightly alkaline, reaction in order that the excess of the powerful

hydrogen ions shall not lead to abnormal changes; and lastly, as we have seen, it must contain such metallic ions as sodium, calcium and potassium in normally balanced proportions.

But Ringer went yet further with the analysis of these effects of electrolytes. For he made the interesting and important observation that the calcium of his perfusion fluid could be replaced by an equivalent quantity of strontium without destroying the efficiency of the salt mixture in maintaining the heart beat. It is evident therefore that it is not calcium as such which is necessary to the tissue, but that it is the effect of some property common to the calcium and strontium ions which is required for the maintenance of normal activity. Barium was found to be a much less suitable substitute for calcium than strontium—a circumstance not to be wondered at when we remember that of this group of closely related metals barium shows less general chemical resemblance to calcium than does strontium.

In a similar kind of way it is found possible to replace the sodium basis of the perfusion fluid with lithium and its potassium by the related rare element rubidium, and to a less extent by cæsium.

Now it is this possibility of replacement of one ion by another which gives us our clearest hint as to the way in which these ions exert their activities in living tissues. For not only do we find that they can be arranged in series showing a regular gradation of general chemical properties, and a corresponding gradation of physiological action, but we also find that similar series will express the several powers which the ions possess of affecting colloidal systems by causing precipitation and such-like changes.

Let us quote an example. It has been found that the cilia of the frog's œsophagus soon became damaged and remain motionless when placed in a pure sodium chloride solution. They thus show a behaviour similar to that of the frog's heart. In a solution of potassium chloride they are preserved for a longer period, but even so they eventually die. On the other hand, in a solution of a lithium salt the cilia remain active for an even shorter time than in pure sodium chloride. If in a similar way we examine the rarer alkali metals, rubidium and cæsium, we find that the several ions sort themselves into a series thus:

$$K > Rb > Na > Cs > Li$$

in which they are arranged in the decreasing order of the length of time for which they will maintain the ciliary movement. Potassium is therefore the least, and lithium the most poisonous ion.

If now we examine the relative effects of these same ions in the precipitation of colloidal proteins—neutral egg white, for example,—we find that the order of their powers to bring about precipitation (as measured by the smallest concentration necessary to produce a visible effect) is found to be

$$K > Rb > Na > Cs > Li:$$

where lithium produces the least, and potassium the most precipitation.

We thus see that both in the case of the dead egg-white and of the living ciliated cells the ions come to be arranged in the same order.

From facts like these we conclude that the ions necessary for the maintenance of the normal activities of living matter are essential in virtue of the effects they produce

on the colloidal protein and other constituents of that matter. And since it is the electric charge carried by an ion which is foremost in producing changes in the colloidal systems, it is only to be expected that the more specific chemical characters of a particular ion should play a secondary part in its action on living matter. When we find that a strontium ion can play the part of a calcium ion in providing a suitable medium in which the heart can beat we naturally conclude that it is the property common to both—namely, the possession of a double positive charge—which is the chief factor at work in each case; and when we find that the effect of a certain number of hydrogen ions can be imitated exactly by trivalent ions in much smaller concentrations—for now each such ion carries three charges instead of one—when we realise from this for how little the actual chemical individuality of the particular ion counts—we are strengthened in our conclusion.

But when asked how these ions act, we cannot at present make more than the general statement that they exert their physiological action by bringing about changes or maintaining certain conditions in the colloids of the tissues which are submitted to their influence; but what those changes and conditions are, and how they are produced and maintained, we shall only know when more far-reaching investigations into the effects produced by salts on colloids and on living tissues shall have been made, and a more searching comparison between the results of these two lines of study shall have been instituted.

BIBLIOGRAPHY.

In addition to the general treatises of Bayliss and Höber, to which we referred in the bibliography to chapter XVIII., we would recommend to the student the following:

For an introduction to the study of colloids, the two excellent little books by Emil Hatschek entitled:

An Introduction to the Physics and Chemistry of Colloids.

Laboratory Manual of Elementary Colloid Chemistry. (London: J. & A. Churchill.)

For further information on the subject of the reaction of body fluids:

The Determination of Hydrogen Ions, by W. M. CLARK. (Baltimore: Williams & Wilkins Company.)

Die Wasserstoffionenkonzentration, by L. MICHAELIS. (Berlin: Julius Springer.)

For a biographical account of Ringer's investigations on the functional importance of salts:

In Memory of Sidney Ringer, by B. MOORE. (*Biochemical Journal*, Vol. V. (1911), p. i., appendix.)

Index

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